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LACTOBACILLUS ACIDOPHILUS

Courtesy of Henri Manuel, Paris

ÉLIE METCHNIKOFF

LACTOBACILLUS ACIDOPHILUS

BY

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COMPOSED AND PRINTED AT THE
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TO
MY PARENTS AND THE MANY INVESTIGATORS,
KNOWN AND UNKNOWN, WHO HAVE MADE
POSSIBLE THIS WORK

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CONTENTS

Preface xi

CHAPTER I

The Antecedents of L. acidophilus Therapy 1

CHAPTER II

A Critical Review of L. acidophilus Investigations 11

CHAPTER III

Contributions from Yale University 26

CHAPTER IV

L. acidophilus versus L. bulgaricus Milk Feeding 32

CHAPTER V

Clinical Results

I. Preliminary 39
II. The treatment of constipation 48
III. The permanence of results obtained by *L. acidophilus* therapy 53
IV. The treatment of diarrhea by *L. acidophilus* milk 55

CHAPTER VI

The Influence of L. acidophilus on the Intestinal Flora 60

CHAPTER VII

The Nature of L. acidophilus Therapy 107

CHAPTER VIII

Indican as Influenced by Constipation and L. acidophilus Therapy 117

CHAPTER IX

Practical Recommendations 177

CHAPTER X

General Summary 181

Bibliography 184

APPENDIX

I. METHODS ... 194
  A. Preparation of *L. acidophilus milk* ... 194
  B. Preparation of whey agar ... 195
  C. Equipment ... 195
  D. Dosage ... 195
  E. Large scale production ... 196
  F. Fecal analysis ... 196
II. SOME TEMPERATURE STUDIES ON L. ACIDOPHILUS MILK ... 197
III. THE USE OF MODIFIED GRAM STAINS ... 203

INDEX OF AUTHORS ... 207
INDEX OF SUBJECTS ... 210

ILLUSTRATIONS

Élie Metchnikoff..*Frontispiece*
Flask of *L. acidophilus* milk (plate 1).................................. 20
Whey agar plate of *L. acidophilus* colonies (plate 2).................. 52
Single *L. acidophilus* colony (magnified) (plate 3).................... 84
Gram-stained *L. acidophilus* (plate 4)................................ 116
Average urinary acidity in *L. acidophilus* treatment (fig. 1).......... 171

PREFACE

Sour milk therapy, as formulated by Metchnikoff, has undergone various modifications. None of these are so striking as the development of *L. acidophilus.* Yet it may be truthfully said that while considerable progress has been made along experimental lines, clinical results have lacked definition. The present volume, therefore, is an attempt to reduce clinical observations to quantitative terms. By so doing, it is hoped that conclusions may be arrived at objectively rather than subjectively.

The most difficult task of all is to adequately express appreciation of the assistance received in the course of this work. It is a privilege to acknowledge my indebtedness to Dr. G. H. Kirby, Director, and to all the members of the staff of the Psychiatric Institute; particularly Dr. C. B. Dunlap, Neuropathologist, for constant and invaluable advice. My assistant, Mr. Philip Beerman, has not only performed most of the laboratory work, but has advanced many important suggestions. Special thanks are due Dr. Leo F. Rettger, Professor of Bacteriology at Yale University, and his colleagues for their timely aid and interest. *The Journal of the American Medical Association, Archives of Internal Medicine, Journal of Infectious Diseases, Journal of Metabolic Research, Journal of Laboratory and Clinical Medicine,* and *State Hospital Quarterly* (*N. Y.*) have very kindly permitted me to reprint material.

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NICHOLAS KOPELOFF

New York, April 15, 1925

CHAPTER I

THE ANTECEDENTS OF L. ACIDOPHILUS THERAPY

Man's first drink is milk. Moreover, it remains a staple article of diet throughout his life. "The perfect food" has been subjected to much ingenious modification that it might tickle the diverse palates of the human beings who populate this earth. The souring of sweet milk was one of the first and simplest methods to be employed extensively and even the Bible makes frequent mention of its use. Thus, "soured milk of kine and goat's milk, with fat of lambs and rams of the breed of Bashan" (Deut. xxxii: 14) are named by Moses as being among the foods given to his people by Jehovah. In Egypt, "leben raib," which is nothing more than a soured milk prepared from the milk of buffaloes, cows, or goats, has been in use since antiquity. Many tribes in Africa have similar tastes. Products more familiar are the koumiss of the nomadic tribes of Asiatic Russia, kefir of the Caucasus and matzoon of the Armenians. Perhaps the best known preparation is the yoghurt of the Balkan Peninsula.

The popularity of the latter is due chiefly to the writings of Metchnikoff (119) and his followers, Combe (47), and others. Believing that many human ills arise from harmful bacteria present in the large intestine of man and further that senility is causally related to this "useless" portion of the gastro-intestinal tract, Metchnikoff arrived at the conclusion that a favorable intestinal flora was the *summum bonum*. He attributed the long life of the Balkan peasants to their widespread use of soured milk which yielded the now famous *Lactobacillus bulgaricus*.

Metchnikoff's next logical step was to inoculate sterilized milk with pure cultures of this bacillus and note its effect after ingestion. He then became convinced that by transforming the intestinal flora he had found a practical solution to an important problem and that "agents which arrest intestinal putrefaction must at the same time postpone and ameliorate old age." The experimental data which form the basis of such a conclusion obviously require the closest critical scrutiny. Unfortunately, a number of contributions in this field scarcely merit such consideration, in that they represent opinion or observation unaccompanied by actual findings. Consequently, for the present purposes, there would seem to be little point in compiling a complete bibliography such as may be found elsewhere; rather a detailed discussion of the more important publications will be attempted.

First, we may quote Grigoroff (72) who isolated *L. bulgaricus*, from "kisselo-mleko" naming the organism "Bacillus A," and describing it as follows:

> A long rod, non-motile, Gram-positive, occurring singly or in chains. It does not grow at all, or poorly, in ordinary nutrient media but on the contrary develops rapidly in all media containing sugar. Glucose broth is uniformly turbid in 24 hours and a whitish sediment is deposited. In 3 to 4 days the supernatant liquid clears. The bacillus ferments glucose as well as lactose. It does not grow on potato or lactose gelatin. It is a facultative anaerobe and does not produce indol. Milk is coagulated in 10 to 14 hours at 37°C. due to the formation of lactic acid. This bacillus is a *true denitrifier*. At 60°C. it is killed in about one hour. It attacks mannite, sucrose, maltose and levulose; but not rhamnose, dulcite or sorbite.[1]

It was to Cohendy, his confrere at the Pasteur Institute, that Mechnikoff turned for his experimental results. In a series of notes published in 1906 the following facts were recorded (42), (43): Two men and two women were fed daily 250 cc. of a 24 hour milk culture of *L. bulgaricus*. This organism was recovered from the feces in considerable numbers from the third to the fifth day after ingestion as well as every day thereafter for 24 days. Prior to this administration, no *L. bulgaricus* occurred in any of the fecal specimens. It was found that not only were the volume and weight of the feces increased but that they became either slightly acid or neutral in reaction. The strain thus employed required 8 days for implantation and persisted for at least 12 days after treatment was stopped. A subsequent report (44) deals with results obtained on himself over a period of 5 months and on thirty subjects receiving 250 cc. of *L. bulgaricus* milk for from 10 days to 7 months.

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[1] All material quoted from languages other than English is the author's translation.

> Immediately after the acclimatization of the lactic organism in the colon, that is to say, after the seventh day of ingestion of a pure culture, the disinfection of the intestine becomes evident. It is manifested at first by the functional modifications observed, namely a very notable deodorization of the stools together with a more easy evacuation. Also there is a reduction of almost two-thirds of the sulfo-conjugates into sulfates.

Thirty grams of sugar were given daily to each patient but this did not appear to be necessary. It is claimed that the "disinfection" established above persists for two weeks after administration has been stopped. Finally, Cohendy (45) attempted to treat acute muco-membraneous enteritis by implanting *L. bulgaricus* in the large intestine. Four out of the five subjects were benefited by such treatment by the eighth to tenth day. The fifth subject, who did not improve, had had the upper two-thirds of the large intestine removed by surgical operation. This further emphasized that where benefit was obtained, it was due to actual implantation.

Analysis of the foregoing results is extremely difficult because the actual data published are so meagre. Undoubtedly Cohendy had all the evidence necessary to establish the important points made, but the impartial critic is deprived of the opportunity of considering the intermediate steps and must of necessity confine himself to the conclusions. Questions naturally arise which are germane to the problem of whether *L. bulgaricus* can be implanted in the human intestine. In the first place it would be of importance to know what were the relative percentages of *L. bulgaricus* found during treatment and how complete was the final implantation? The question on which clinicians so much desire information: whether during treatment there is a greater frequency of evacuation was not answered in quantitative terms. No mention is made of the actual numbers of viable *L. bulgaricus* fed per cubic centimeter of culture and in view of the fact that implantation appears to be a phenomenon of mass inoculation, this might not be insignificant. Further, the question might be raised as to whether there is actual implantation when the organisms persist for only 2 weeks after administration. Finally, may not the ingestion of 30 gm. lactose daily by these patients have stimulated in the colon a growth of *L. acidophilus* indistinguishable on the plates from *L. bulgaricus,* thus confusing the results? If one is forced, then, to judge the conclusions of Metchnikoff by the published notes of Cohendy, their validity is doubtful.

The effect of *L. bulgaricus* in the intestine was studied by another of Metchnikoff's colleagues, Belonovsky (29) who used mice as experimental animals. In a well-planned investigation one set of mice were fed sterilized grain with a one to two day old *L. bulgaricus* milk culture added; another set received grain and sterile milk; a third set received grain and sterile water; a fourth set received grain and milk coagulated by lactic acid in amounts equal to that present in the *L. bulgaricus* culture; a fifth set received grain with *L. bulgaricus* killed at 56°C.; a sixth set received grain inoculated with *B. prodigiosus;* and a final set received grain with *B. pyocyaneus.* Implantation of *L. bulgaricus* occurred in 10 days and remained for as long as 2 months thereafter. In the average bacterial content of feces after treatment the mice fed on *L. bulgaricus* showed the lowest count. These animals likewise proved to be the heaviest in weight. Studies *in vitro* soon showed the predominance of *L. bulgaricus* over other organisms. However, pasteurized cultures of *L. bulgaricus* and *L. acidophilus* did not contain a substance as toxic as that observed in living cultures. Belonovsky concludes, therefore, that the beneficial effects of *L. bulgaricus* in the intestine are not due solely to the lactic acid produced by this organism. Again, the author does not give more detailed findings. There is no statement of the number of mice employed in this experiment, a fact which seriously influences the value of the conclusions. There were, to be sure, at least two animals in each set, but information is wanting with regard to the agreement or variation between the bacterial counts from each individual. Not infrequently the differences in bacterial counts between individuals receiving the same treatment is greater than the differences between the average counts of different treatments. Further, no mention is made of the amount of culture fed to these mice, nor of the number of viable organisms present.

For further corroboration of the favorable results obtained with *L. bulgaricus* it is necessary to turn to German publications. Wejnert (183) arranged an investigation in which ten subjects received sour milk administered rectally; eight subjects received an ordinary diet reinforced by sour milk administered orally; and eight subjects received the usual diet reinforced by *L. bulgaricus* milk. There is given a very satisfactory account of the individual subjects, including diet and bacterial counts of feces. Two to three bacteriological analyses of feces were made prior to the special treatment administered and no lactobacilli noted. In a majority of instances the bacterial counts of the feces were reduced after treatment. The count returned to its high level within 3 to 4 days after the feeding of sour milk was stopped. However, where "Metchnikoff milk" was administered the reduction in bacterial counts was much more striking and returned to normal much more slowly after the treatment was discontinued. Thus, after 10 days they had not yet returned to one-half the normal number. Some clinical improvement was likewise noted in these cases. This was accompanied by a change in color of the feces. This interesting contribution is rather limited in scope. No mention is made of the actual numbers of *L. bulgaricus* administered or found, so that the actual extent of implantation remains questionable. Furthermore, if the feces were not weighed and no due allowance made for variation in the moisture present in different samples, the total bacterial counts of feces can have slight significance. Consequently, it adds little conclusive proof to the evidence advanced by Metchnikoff's colleagues.

A somewhat less satisfactory paper is that of Leva (111). He reports little change in the urinary indican of patients who received "lactobacillin" in addition to their regular diet. However, he states that he found *L. bulgaricus* after 5 days, in his own stools, as well as in the stools of fifteen patients who had taken yoghurt milk. Good clinical results were also claimed. However, no actual findings are presented, and no comparison is made of the bacterial content of the patients' stools, before and after treatment.

The investigations tending to disprove the claims of Metchnikoff are, with certain exceptions, no more convincing than those in his support and suffer from the same experimental deficiencies. Luerssen and Kühn (114) themselves ingested one-third of a litre of *L. bulgaricus* yoghurt daily. Prior to this feeding, they found no *L. bulgaricus* in their feces. After 14 days Kühn found *L. bulgaricus* "sparingly." Only a few organisms were found 3 days after discontinuing treatment and none after 5 days. Luerssen found a very few *L. bulgaricus* in his feces after 17 days and none 5 days after discontinuing treatment. They repeated the experiment, using a yoghurt containing 3 strains of *L. bulgaricus* but again 5 days after feeding no living organisms were recovered. Their conclusion was that implantation was impossible. However, their data seem too meagre for such a sweeping generalization.

Oehler (131) claims to have found *L. bulgaricus* in three different kinds of yoghurt but no viable organisms in dry preparations such as powders, tablets and pastilles. After feeding *L. bulgaricus* to mice and monkeys, the organism was recovered from the feces. There was neither any demonstrable changes in flora nor any real implantation, for the organism disappeared from the feces from 2 to 3 days after the last feeding. Since no data are submitted, little value can be ascribed to the conclusions.

"Lactobacilline," the product produced by a Paris concern reputed to be under Metchnikoff's supervision, has been used extensively. Klotz (97) fed it to fifty children suffering from severe summer complaint, of which number thirty-one died, notwithstanding. In his analyses of the feces he claims to have found no reduction in the counts of *B. coli*. His conclusion was that *L. bulgaricus* therapy was of no value. However, the lack of data does not warrant such a generalization.

Severin (158) discussing the differences between various milk-souring bacteria sounded the timely warning: that little was known concerning the *L. bulgaricus* found in "Lactobacilline" as compared with other lactic acid organisms.

Distaso and Schiller (56) fed white rats *L. bulgaricus* cultures with lactose and noted a predominance of *L. bifidus*, with *L. bulgaricus* present after 24 hours. The number of the latter diminished rapidly thereafter. The experiment was repeated with 10 cc. of milk as the pabulum for *L. bulgaricus*, with identical results. The conclusion reached was that foreign bacteria do not become acclimatized to the intestinal tract. Besides the fact that no data are presented, the obvious criticism here is that the feeding of lactose undoubtedly stimulated other lactobacilli, particularly *L. acidophilus*, thereby unduly "loading" the results against *L. bulgaricus*.

Kulka (109) administered *B. prodigiosus* and *Vibrio Metchnikoff* orally to human subjects and attempted to recover these organisms from their feces without success. He concluded that it was impossible to obtain an excretion of "alien" bacteria.

Herter and Kendall (77) in an experiment with a single monkey, found that daily feeding for 2 weeks of 500 cc. of *L. bulgaricus* milk exclusively "failed to establish the predominance of this organism in the ileo-caecal region or in the large intestine. In the latter situation the number of bacilli of this type was relatively small and decreased towards the anus. Thus in the regions characterized by most active putrefaction the lactic acid bacilli failed to establish themselves in relatively large numbers." The actual number of viable organisms fed, or the number found in the intestine and feces is not mentioned. Nor is it stated for how long after feeding, *L. bulgaricus* could be recovered.

Rahe (138, 139), an American, was the first investigator to present adequately the results of a critical investigation of *L. bulgaricus* implantation. Broth and milk cultures of *L. bulgaricus* were fed in large amount to three human subjects for 26 to 44 days. The first appearance of the organism in the feces was noted as well as the maximum numbers attained and numbers subsequently recovered. In the first subject, although the greatest elimination of *L. bulgaricus* occurred on the day after feeding was stopped, no *L. bulgaricus* were found during the following 5 days. In the second subject the organism occurred in maximum numbers on the sixth day, but disappeared on the third day after the cessation of feeding. The same failure was noted in the third subject in the same period of time. In still another subject who had received broth cultures of *L. bulgaricus* for 8 weeks, the organism was recovered 8 days after discontinuing the administration. No statement is made concerning subsequent examination, so that the importance of this finding is rather difficult to evaluate. It is undoubtedly significant in so far as it contradicts the findings in the first three subjects studied and it is to be regretted that the author has failed to discuss this case more fully, if only on that account.

Three monkeys were also fed *L. bulgaricus* and killed at different intervals after treatment was discontinued. In one monkey fed about 10,000 *L. bulgaricus* per day, no organisms could be recovered 24 hours after the last feeding except in the jejunum. In another monkey, killed 7 hours after the last ingestion of milk culture *L. bulgaricus* was found in all portions of the intestinal tract. In another monkey killed 7 days after the last ingestion, *L. bulgaricus* was found only in the duodenum. No records were published of fecal examinations of these monkeys during treatment. Rahe concludes that *L. bulgaricus* "cannot become adapted to the human lower intestine. The experiments with monkeys show that the *bacillus bulgaricus* is capable of an apparently limited survival in the upper intestine of these animals."

Such work carries more conviction than any publication previously surveyed. Obviously the conclusions must be limited in value in the light of the material presented. There are only four human subjects and three monkeys involved. However, the experiment appears to be carefully controlled. There still remain to be mentioned the important contributions from Yale University, but the high calibre of the investigations and the completeness with which the data are presented justifies a separate consideration which may well be reserved for a succeeding chapter.

While investigations dealing with *L. bulgaricus* may be regarded as the principal antecedents of *L. acidophilus* therapy, another group of studies dealing with the control of putrefaction in the human intestine, have a direct bearing on the subject. In such studies milk and carbohydrate feeding were employed. The composition of the urine excreted under these conditions has generally been considered a measure of putrefaction in the intestine. However, as will be shown later in discussing quantitative determinations of indican in the urine (see Chapter VIII) such an indirect method is open to serious criticism because of the wide range of variation occurring in the same individual from day to day on a uniform diet. Backman (10) gives an excellent review of the literature and agrees with Biernacki (21), Eisenstadt (59), Krauss (108) and others that carbohydrates have little if any influence on protein decomposition in the intestine, though the contrary view is held by Hirschler (81) and his followers. Rovighi (155) found that 1½ litres of kefir per day inhibited putrefaction and considered that this was due in part to the lactic acid formed. Winternitz (185), however, concluded that milk inhibited putrefaction because of its lactose content, and independently of acids formed. Grundzach (73) considered that the alkaline secretion in the large intestine was responsible for the development of putrefying bacteria and that lactic acid hindered this multiplication. His suggestion as to the properties of "an ideal" anti-putrefactive agent is worth quoting. "It would be a substance in itself insoluble, yielding soluble products on disintegration which would be harmless and act as strong acids *in statu nascendi*." Schmitz (156) in a series of patients found that hyperacidity reduced putrefaction. Albu (1) believed that lactose exerted an inhibitory action on putrefaction by stimulating peristalsis and by certain "antiseptic" effects. Herter (75) introduced lactic acid bacteria into the intestine and noted a marked reduction in urinary indican and ethereal sulphates. Later Herter and Kendall (78) in experiments on kittens and monkeys found that an abrupt change in diet from one predominantly protein in nature to a milk and sugar regimen was followed by an alteration in: (*a*) the nature of the intestinal flora; (*b*) putrefactive products of feces and urine; and (*c*) clinical condition. They concluded that:

> The chief character of the bacterial change is the gradual but rapid substitution of an acidophilic, non-proteolyzing type of flora for a strongly proteolytic type. The chief feature of the putrefactive conditions in the intestine is the reduction of indol, skatol, phenol and bound hydrogen sulphide and a diminution in the indican and aromatic oxyacids of the urine. Clinically the most striking feature of the change of diet is (in monkeys) an improvement in spirits and activity which may safely be construed as showing a markedly improved sense of bodily and physical well-being.

It appears therefore that the influence of milk and carbohydrates on the intestinal flora was a moot question before *L. acidophilus* therapy was introduced. Obviously it would be hazardous to base any conclusions in this complex field on any single indirect determination such as urinanalysis, particularly when information as to "normal" values is so sadly wanting. However, even from this brief review it will be noted that there is sufficient evidence to warrant further research along these lines. Presently it will be seen that this has gone forward.

To recapitulate briefly: it is evident that the question of the implantation of *L. bulgaricus* in the human intestine is the crux of the whole problem of *L. bulgaricus* therapy. The claims made by Metchnikoff are none too convincing when the experimental data are carefully analysed. Similarly, his critics furnish a paucity of substantial evidence. In fact, one is led to believe that the whole matter belongs far more to the category of speculation than to that of scientific verity.

CHAPTER II

A Critical Review of L. acidophilus Investigations

Inconsistent as the results of *L. bulgaricus* therapy have been, they have served to stimulate investigations dealing with the intestinal flora and its simplification. Paradoxically enough, the discovery of *L. acidophilus* actually antedates that of *L. bulgaricus* by about 5 years. Moro (123) in 1900, describes the organism he named "Bacillus acidophilus" as follows: In stool preparations, young cultures, etc., it is a strongly Gram-positive rod 1.5 to 2 μ long, 0.6 to 0.9 μ wide, with slightly tapering ends. In acid beerwort bouillon or other acidified fluid media a finely flocculent sediment is formed, leaving the supernatant liquid clear. If not transferred the bacillus loses its power to take the Gram stain in from 36 hours to 9 days and the rods become longer and thinner. After a week degenerative forms occur. The colonies on plates vary in appearance according to the depth at which they grow in the medium. Surface colonies have a delicate tangled, hair-like periphery and become denser towards the center. The deeper-lying colonies show the characteristic branching threads only sparingly. Frequently one can see round and other forms without threads. Smears from colonies give the characteristic microscopic appearance. On agar slants (beerwort, sugar, glycerin, blood serum) growth is very slight and circumscribed, occurring as a dry veil over the streak which seems to be composed of tiny streptococcus-like colonies. The optimum temperature for growth is 39°C. No growth can be seen at 20° to 22°C. In milk, growth occurs slowly, taking about 3 weeks for complete curdling. There is no growth on potato. The bacillus forms no gas. It is active in the production of acidity (dextrose bouillon). Better growth is obtained where oxygen is excluded, but it is not an obligate anaerobe. The bacillus has no flagella. It is non-pathogenic for animals. The bacillus is able to withstand the high acidity of the feces of breast-fed children and can always be isolated from this source.

It was pointed out by Kendall in 1910 (94) that the term "acidophilic" for *L. acidophilus* and similar organisms is essentially a misnomer, because the bacteria in this group *endure* acid rather than *love* it. Therefore, he suggested that "aciduric" would be a more satisfactory characterization. Subsequent investigators in this field have, generally speaking, adopted this nomenclature.

In the same year that Moro discovered *L. acidophilus* Finkelstein (61) and Tissier (170) confirmed his findings. The latter describing the flora of nurselings states: "One finds the bacillus described by Moro in the stool of infants receiving a mixed diet, sterilized milk, and above all ordinary milk. It occurs similarly in diarrheas." However, Tissier (170) contended that his *B. bifidus* was the predominating organism in the stools of breast-fed babies, and not *L. acidophilus* as claimed by Moro (124). This was confirmed by Cahn (28), Rodella (152), Passini (134), Jacobson (89), Bahrdt and Beifeld (11), and admitted at a later date by Moro (125). Sittler (162) found no great differences between the intestinal flora of children fed on breast milk or on cows' milk. He claims to have induced a *B. bifidus* flora by feeding lactic acid, bacillin, etc., but no quantitative results are reported.

Wollstein (186) working with infants, found that "the presence of *B. acidophilus* at a time when the reaction of the feces was neither amphoteric nor alkaline to litmus, may be accounted for by the fact that this acid-loving bacillus really grows better in a slightly alkaline medium, a fact pointed out by Rodella (151)." After feeding *L. bulgaricus* to three children, Wollstein claims to have recovered the organism in the stools. No counts are reported and nothing is said concerning the differentiation of this organism from *L. acidophilus*, which may have also been present.

It is scarcely necessary to catalog all the investigators who make mention of the occurrence of *L. acidophilus* in the stools of infants. Logan (113) gives an excellent bibliography in his comprehensive studies on the flora of infants and young children. He concluded that breast-fed children yielded *B. bifidus*, while *L. acidophilus* was found chiefly in artificially fed children. *In vitro* he found that the latter organism restrains Morgan's No. 1 Bacillus and *B. coli* in broth to which lactose or glucose has been added.

L. acidophilus has not only been recovered from the stools of infants but from those of adults as well. Both Cippolina (39) and Weiss (182) reported such findings soon after Moro's original publication.

The investigations up to this point have, historically and scientifically speaking, but formed the ground plan for the more interesting and important structure to follow. Chronologically, it is here that the increasingly important series of contributions of Rettger and his associates first makes its appearance. In 1914–1915 comparative studies of *L. bulgaricus* and *L. acidophilus* in rats were reported, but their experimental work on man was not published until 1920.

In fact, credit is due Torrey (171) for first demonstrating the importance of *L. acidophilus* in the feces of adult human beings and for inducing the transformation of the intestinal flora by means of lactose feeding. Rotch and Kendall (154) had previously suggested the therapeutic possibilities of *L. acidophilus.* Coleman (46) working with Torrey, further indicated its use in the tympanites and diarrhea of typhoid fever. In studying the correlation of conditions and changes in the fecal flora of human subjects with exact data in regard to the diet utilized, Torrey through a painstaking and comprehensive experiment, obtained some significant results. Dealing with typhoid fever patients he found that the addition of 250 to 300 grams of lactose was responsible for a simplification of the intestinal flora establishing *L. acidophilus* as the predominant organism. He obtained similar results (172) in subsequent feeding experiments with dogs. In a comparative study of sugars, lactose and dextrin proved most favorable in transforming the intestinal flora to the aciduric type. Saccharose, maltose, and glucose were less effective, as was milk alone. However, the addition of bread to a pasteurized milk diet resulted in an aciduric flora. While acid glucose agar or oleate glucose agar had heretofore been generally recommended for the cultivation of *L. acidophilus,* Torrey (173, 175) found these media unsatisfactory for the quantitative determination of these organisms in fecal specimens and devised a glucose liver agar for this purpose.

In a recent study, Torrey and Kahn (174) obtained some suggestive results. They demonstrated that *L. acidophilus in vitro* was able to inhibit the usual proteolytic activities of certain spore-bearing anaerobes and it appeared that this phenomenon was entirely dependent upon the acid produced by *L. acidophilus*. Comparative tests with *B. coli* showed that in general there was little difference between it and *L. acidophilus* in inhibiting proteolytic activity. This again stresses the rôle which acidity plays. While the authors have recognized the limitations of work done *in vitro*, they have not considered the question of the influence of amount of inoculum as affecting proteolytic activity. While the amount of inoculum as stated consisted of 0.5 cc. of a 24-hour culture, no statement is made of the number of viable organisms per cubic centimeter. Since differences might readily occur, it it is not inconceivable that they might prove responsible for divergent results until maximum numbers had been reached in the medium.

The intestinal flora in mouse typhoid infection has been studied by Webster (181). He found that in the normal flora of laboratory mice fed on bread and milk, *L. acidophilus* and *B. bifidus* outnumbered *B. coli* and *B. acidi-lactici*, 25 to 1. However, when these animals were infected with mouse typhoid the fecal flora did not change, but remained the same as in animals which resisted infection. Furthermore, mice fed on a meat diet and revealing a predominantly colon flora did not differ in susceptibility to this disease to any greater extent than normal mice fed on bread and milk having a fecal flora containing *L. acidophilus*. Regardless of how interesting the results of animal experimentation prove to be, it is a far cry to infer that they will be duplicated in human beings. Further verification is required.

The same limitations are expressed by Sisson (161) who likewise arrived at negative results in a careful experiment with puppies. Eleven were fed pasteurized cow's milk with the addition of 10 per cent of lactose; four with the addition of sucrose, and five served as controls. The addition of protein milk also had no effect on the intestinal flora.

> That acidophilic organisms. . . . never were in predominant numbers is shown conclusively by the microscopic findings as a smear made up chiefly of Gram-positive bacilli was never encountered. In puppies the types of organisms occurring at the duodenum, ileum, cecum, and

> rectum are in all instances similar. One cannot speak of a characteristic local flora occurring in these regions.

Three infants were examined by means of the duodenal catheter and the flora at various levels proved to be essentially the same. De Gaspari (54) found that rats, on ordinary diet, harbor *L. acidophilus* in their feces, but that with the addition of large quantities of meat their number was reduced, no data being offered in substantiation.

The first published attempt to feed *L. acidophilus*, although it proved unsuccessful, was made by Fisher (63), who also carried on comparative studies with *L. bulgaricus* and *B. bifidus*. After demonstrating that *L. bulgaricus* proved germicidal *in vitro* to three members of the colon group of bacilli, 2 to 4 quarts of *L. bulgaricus* milk were fed to 5 patients for 28 days. Constipation was relieved and a predominantly Gram-negative intestinal flora transformed into one with a large number of Gram-positive organisms.

> Attempts were made to recover *Bacillus bulgaricus* from the feces. The results were unsuccessful in practically all cases. Several times Gram-positive organisms resembling *Bacillus bulgaricus* were isolated. These organisms answer the description of the *Bacillus acidophilus* of Moro. They are very similar to *Bacillus bulgaricus*, but do not form acid as rapidly nor is the maximum acidity as high. The principal point of difference is that *Bacillus acidophilus* can readily be recovered from the feces, while *Bacillus bulgaricus* cannot.

Unfortunately the data presented are not strictly quantitative so that their evaluation is extremely difficult. The viable content of the milk fed is extremely important, particularly since it was stored in a refrigerator before feeding. It has been demonstrated that this practice kills off many viable organisms (102). Again in the light of the later work of Rettger and his associates, and others, it will be seen that the milk acidity test is not at all a dependable criterion in the differentiation of these lactobacilli. But the conclusion arrived at by Fisher stands or falls by the reliability of this test. Continuing along the same lines, this investigator fed sweet whole milk to six patients for 28 days. The results were the same as those obtained while feeding *bulgaricus* milk, proving that the latter organism could not be credited with changing the intestinal flora. He then fed watery suspensions of *L. acidophilus*, *L. bulgaricus* and *B. bifidus*, but no striking benefits were observed; the conclusion being that the presence of milk was in some way associated with the transformation of the intestinal flora. Therefore, Fisher fed lactose (150 to 215 grams per day) to six patients and concluded:

> The feeding of lactose changes the intestinal flora from a Gram-negative one to one in which considerable numbers of Gram-positive organisms of the *Bacillus bifidus* and *acidophilus* types are present. This in connection with a slight decrease in decomposition products seems to point out that lactose is an important factor as a controlling agent in intestinal putrefaction.

But by his own data these conclusions are seen to rest on unsubstantial evidence. A careful analysis of his Table VII reveals the fact that in only one case out of six is there any difference recorded in the intestinal flora before and after treatment, and in this single case the difference is not so marked as when sweet whole milk was fed. Constipation before and after treatment is identical in all six instances. The slight decrease in decomposition products is obviously of little significance. Finally, through this entire investigation no mention is made of more than a single fecal analysis on the same subject before or after treatment; while the period during treatment has been apparently altogether neglected.

In an interesting series of papers, Cannon (29) employing the strictest laboratory methods corroborated some of the conclusions of previous investigators. He found that grain foods, lactose and dextrin fed to rats led

> to a marked predominance of aciduric bacteria in the intestinal tract, whereas animal proteins encourage the gas-producing proteolytic types, both aerobic and anaerobic. Vegetable proteins and certain starchy foods do not encourage the development of proteolytic types to the same extent as animal proteins, and, in fact, in many cases exert a distinct anti-putrefactive effect, favoring the development of *B. acidophilus* and suppressing the development of hydrogen-sulphide-producing organisms and spore-forming anaerobes. In two experiments with human adults extending over a period of 10 days a diet composed of bread, milk and lactose markedly encouraged the development of the aciduric organisms, and in one experiment of the same time period a diet high in vegetable protein led to a predominant aciduric flora with the elimination of anaerobic spores.

His findings have been summarized, in this and succeeding papers, in such a way as to leave no doubt as to the soundness of his deductions.

Dragstedt, Cannon, and Dragstedt (57) found that intestinal stasis or complete obstruction led to the development of a proteolytic intestinal flora irrespective of the character of the diet, and that the intestinal secretions did not suppress the growth of Gram-positive aciduric organisms from closed intestinal loops. They concluded that the disappearance of Gram-positive organisms from closed intestinal loops was probably due to the absence of utilizable carbohydrate and to the alkaline reaction of the medium. In a study of the influence of hydrogen ion concentration on bacterial types, Cannon and McNease (31) found that the actual acidity of the intestinal contents of the cecum and colon of white rats was an important factor in the simplification of the intestinal flora. A pH of 7 0 is regarded by them as characteristic of a gas-producing proteolytic type of flora, whereas an increasing acidity is characterized by a diminution of proteolytic types and their replacement by aciduric types mainly dominated by *L. acidophilus*. Finally, Cannon (30) made a comprehensive biologic study of sixty-four strains of *L. acidophilus* and thirty-four strains of *L. acidophil-aerogenes* classifying the organisms according to Rahe (140). He found that "when grown in dextrose yeast broth in mixed culture with *B. coli* with an initial reaction of pH 7.0 to 7.6, *B. coli* at first gains the ascendancy, but *L. acidophilus* becomes predominant as the pH approaches 5."

The results of the therapeutic application of *L. acidophilus*, first reported in detail by Rettger and Cheplin (144), have stimulated a number of interesting investigations. Gompertz and Vorhaus (69, 70) in a clinical study administered *L. acidophilus* to fifty cases of chronic constipation over a period of about 5 months. Satisfactory results were noted in forty cases. "Of the chronic diarrheas, again only the severest cases were tested, and to date 10 cases out of 12 have shown marked improvement." Finding the administration of milk cultures impracticable, a broth culture containing 100 million *L. acidophilus* organisms per cubic centimeter was given in from 10 to 20 cc. doses two, three, or four times a day before meals. Bacteriologic and microscopic examinations confirmed the findings noted by Rettger and Cheplin

(144), namely a transformation from a Gram-negative to a predominantly Gram-positive intestinal flora. In a later communication Gompertz and Vorhaus (71) claim that in the treatment of 200 patients suffering from auto-intoxication with chronic constipation:

> Seventy per cent showed complete relief from their symptoms of toxemia; 15 per cent showed some improvement; while the remaining 15 per cent showed none whatsoever. Since the 15 per cent of failures included many who did not continue the culture for an adequate length of time, we feel reasonably sure that if this treatment is maintained for 5 weeks in every case a larger percentage will show an improvement. In contradistinction to the relief of the symptoms, the constipation itself showed complete relief in only 25, improvement in 35, and failure in 40 per cent of the cases. In the second group, the diarrheas, there was complete relief from all symptoms in 70, some relief in 10, and failure in 20 per cent of the cases (100). Although this study has not been carried on long enough to determine the permanence of these figures, we wish at this time to record the fact that, in those cases under observation for a year or more, from 10 to 15 per cent showed a return of their symptoms.

This array of clinical material supported by laboratory findings is assuredly impressive. While one could scarcely question the validity of the final statistics, nevertheless it is unfortunate that no other quantitative data are published. This is particularly true where the interpretation of results is so largely a personal one. In the last analysis "auto-intoxication" and "constipation" are a matter of definition, consequently what one investigator might refer to as "relief," another working on the same clinical material might conceivably call "improvement." Obviously, this example would be far-fetched in certain striking instances usually cited as illustrative, but would apply to a large number of milder cases. Finally, the absence of any bacteriological data robs such an important contribution of many interesting points.

The same general comments would also be applicable to the interesting reports of Norman and Eggston (130), Bass (12, 13, 15, 16), Pope (136), Reddish (141), Mizell (121), Clark and Perry (40), and Jimenez (5, 90). All of the above named investigators have been successful in the clinical application of *L. acidophilus*. But as with the one-time favorable reports concerning *L. bulgaricus*, they are interesting, but scarcely objective or detailed enough to satisfy scientific doubt or critical analysis. A new technic in the preparation of *L. acidophilus* has been devised by Eggston and Norman (58) using unsweetened evaporated milk and water. Bass (14) recommends pasteurized rather than sterilized milk for large-scale production. Reddish (141) reported twelve cases dealing with a variety of clinical conditions of constipation, including mucous colitis successfully treated by *L. acidophilus* milk. Mizell (121) working with two groups of ten patients, the first dealing with intestinal toxemia of a putrefactive type, and the second group dealing with chronic colitis and stasis with no putrefactive toxemia used urinary findings as a basis for concluding that 40 per cent of relief from stasis and indicanuria occurred in the first group, and 60 per cent relief in the second group. Seventy-five per cent of both groups had undergone various types of surgical treatment. Clark and Perry (40) claimed that of twenty patients receiving *L. acidophilus* alone, eight became more constipated, six became less constipated, and six were without effect. Of thirty cases receiving *L. acidophilus* and lactose, twenty-four became less constipated and six showed no effect. Twenty-three out of twenty-seven patients complaining of intestinal gas were relieved. One case of ulcerative colitis was unaffected by treatment. Five cases of chronic myocardial weakness were benefited. Good results were obtained with six cases of chronic diarrhea, one case of sprue, and three out of four chronic eczema cases. Of eight psycho-neuroses, four were benefited and four not. The use of lactose after 6 weeks administration of *L. acidophilus* and lactose treatment is recommended. On stopping lactose a "rapid reversal to original type of flora" ensued.

In working with psychotic patients, Julianelle and Ebaugh (92) implanted *L. acidophilus* with results similar to those obtained by Rettger and Cheplin (144) whose technic they followed. They concluded that intestinal putrefaction was eliminated and that in some cases a gain in weight occurred, but that there was no improvement in the mental condition of these patients. Their data have been presented in composite graphs which makes individual evaluation impossible except for the qualitative improvements noted in the case abstracts.

Morriss (126) working in a tuberculosis sanatorium with *L. acidophilus* milk has reported a valuable investigation carefully controlled clinically as well as bacteriologically. He states that "it is our feeling that whatever improvement has occurred in our small series of patients is due probably to some other factor than the actual transformation of the common intestinal bacteria into the *acidophilus* type of organism." Singularly, this investigator is hypercritical of his own work, for the data show relief in all of six cases of diarrhea; six improvements, and five slight improvements in thirteen cases of indigestion; and two improvements and one slight improvement in four constipated cases. The fact that the percentage of *L. acidophilus* recovered from the feces was lower than anticipated may be due to the lack of a sufficiently large number of viable organisms in the dosage, the omission of lactose, and possibly the nature of the diet.

In striking contrast to the above experimental attitude is the generalization indulged in by Bassler and Lutz (17), which remains unsupported by any evidence, to the effect that *L. acidophilus* therapy is of little avail.

In a suggestive study Goldman (68) made frequent intestinal examinations of a patient receiving a special *L. acidophilus* culture. Implantation of this strain was unsuccessful, although better results were obtained when lactose was also administered. Streptococci were the only organisms recovered after feeding a sterile diet and *L. acidophilus* in broth culture. It is unfortunate that the *L. acidophilus* employed in the first half of the work is open to serious question since it did not ferment maltose. It is not unlikely that the organism in question was *L. bulgaricus*, particularly since it came from a commercial rather than a scientific source. The number of viable organisms fed in the second part of the experiment was perhaps too small to be considered adequate for a stubborn case.

The use of *L. acidophilus* in England has recently been reported by Smith (165). After feeding milk cultures of *L. acidophilus* to human typhoid bacillus carriers, it was found that after 4 days there was a transformation of the flora to a predominantly *L. acidophilus* type (80 to 90 per cent of the organisms present in Gram-stained films). Lactose was also effective in causing a similar simplification. The reaction of the feces was found not to be influenced by the administration of *L. acidophalus* milk or lactose. Failure to recover *L. bulgaricus* from feces was noted

Plate I. Flask of L. acidophilus Milk So Inclined as to Show the Smooth Homogeneous Quality of the Curd

Note how the solid curd falls away from the walls of the flask without disintegrating. The clearer supernatant liquid is the whey. The odor and taste of *L. acidophilus* milk is also characteristic.

after feeding one-half litre of a milk culture to three individuals. *B. lactis aerogenes* was not recovered from feces after oral administration until a vaccine had been injected subcutaneously. It is particularly to be regretted that further details of these interesting experiments were not published, so that a critical analysis might be made.

In studying the distribution of intestinal spirochetes in human subjects on special diet, Parr (133) noted that lactose was responsible for a diminution in the percentage of spirochetes present. This investigator also used *L. acidophilus* milk and concluded that it was responsible for a decrease in the number of spirochetes.

> The ratio of colon bacilli present to those of the *acidophilus* type decreased but could be reduced no lower than 16 to 73. Both in the lactose feeding work and in this milk feeding, the spirochetes appeared in the original concentration very soon after stopping the lactose or the milk.

In a more recent communication, Hudson and Parr (86) have adequately presented the results of a very interesting experiment with rats.

> It was found that a diet containing sufficient carbohydrate to effect a complete turnover from a heterogeneous, Gram-negative type of flora in rats fed on high protein and stock diets, to a flora markedly simplified and Gram-positive, induced consistently a change in the H-ion concentration from nearly neutral to distinctly acid. In rats fed on a high carbohydrate diet, this acid environment was much more marked in the cecum than in the colon. . . . . Intestinal spirochetes were found in a large number of rats on a high protein and stock diets, and in the cecum of these rats to a greater degree than in the descending colon, coincident with putrefaction and less acidity.

The ingestion of 250 grams of lactose per day according to Robinson (150) caused an initial diarrhea with a corresponding acidity, but subsequently with the subject's acquisition of a greater tolerance for the sugars, the reaction of the feces returned to normal. Diarrhea was found to cause acid feces whereas constipation tended to make them alkaline. The administration of a quart of *L. acidophilus* or *L. bulgaricus* milk did not change the daily reaction of the feces. Blatherwick and Long (22), however, found that the ingestion of a large amount of lactic acid milk caused the formation of a strongly acid urine, due chiefly to the presence of acid phosphate. Tisdall and Brown (169) found in a study of the acidity of infant's stools, that the hydrogen ion concentration depended on the ratio in the *intestinal zone of fermentation* of the carbohydrates on the one hand to the proteins and bases on the other.

In a careful research on the intestinal flora in diarrhea, Hines (80) found that in eight cases of diarrhea with intestinal lesions the flora was proteolytic. In two cases of fermentative diarrhea, one had an aciduric flora, while the other proved to be proteolytic. It was further observed that spores of *B. welchii* were present in great numbers in feces with a proteolytic flora but were absent in the case of an aciduric flora.

Turning now to the more strictly bacteriological aspects of *L. acidophilus* and its relation to *L. bulgaricus*, it was assumed for a long time that these organisms could be differentiated by the amounts of acidity developed by each in milk. It remained for Rahe (138) to establish that there exists a more reliable criterion, namely, the fermentation of maltose. He found that *L. acidophilus* ferments this sugar, while *L. bulgaricus* does not. In fact, this investigator classified the whole group of aciduric bacteria in a comprehensive and painstaking manner on the basis of sugar fermentation. This work has been generally accepted although investigators have occasionally noted exceptions. Cruickshank and Berry (50), however, make contradictory claims. They found that forty-four out of fifty-two strains of *L. acidophilus* isolated from feces did not ferment maltose, and that four did not ferment glucose. A considerable range in morphology was noted and it was suggested that the term *L. acidophilus* covers a considerable group of organisms; in fact, they believe that numerous strains may exist in the same stool. Since the work of these investigators differs so radically from that of Rahe and his corroborators, it is possible that either the English investigators were not dealing with typical *L. acidophilus* organisms; or that the question of sugar fermentation is still an open one. The latter position might be regarded as sound in view of the exceptions which have been recorded (2, 50) as well as those which are still unpublished. The differentiation of *L. acidophilus* from *L. bulgaricus* remains perhaps the most baffling problem in this field. Until it is definitely settled, the impartial critic will be at a loss to determine whether various investigators have dealt with the same or different organisms: whether therapy has been a matter of chance or plan.

Recently, however, there has appeared a brief report which is both significant and encouraging. Albus and Holm (2) finding that a differentiation between *L. acidophilus* and *L. bulgaricus* on the basis of sugar fermentations "is not absolutely infallible" offer a new criterion, namely, surface tension. They find that all strains of *L. acidophilus* grow very well in a medium of a surface tension as low as 36 dynes, while *L. bulgaricus* in the same medium depressed to a surface tension of 40 dynes fails to show growth after 7 days incubation at 37°C. To quote these authors:

> A surface tension of forty dynes represents the extreme lower limit for *Lactobacillus bulgaricus* as most of our strains were inhibited above this value. This, then, offers a means of differentiating *Lactobacillus bulgaricus* from *Lactobacillus acidophilus* which separated, without exception, all of the strains employed in this work. That surface tension may be a factor in the implantation of these organisms seems plausible.

The following studies with *L. acidophilus* are of interest: Slanetz (163) indicated that the thermal death point for a 48-hour culture is 62° to 63°C. for 10 minutes. Kendall (95, 96) has made extensive studies in the metabolism of this organism and *B. bifidus* but found nothing of striking significance. Breidigam and Chang (25) have reported a device for isolating the organism from single colonies.

Fischer (62) working with *B. coli* concluded that lactose inhibited indol production in *B. coli* by means of a specific property of the sugar, namely, the inactivation of the proteolytic enzymes of *B. coli*. Neither the production of special acids nor the pH was of significance. This suggestive experiment is mentioned merely because it offers a possible explanation of the anti-putrefactive activity of *L. acidophilus* in the human intestine.

Dr. Harper F. Zoller, Chief Chemist of the Nizer Laboratories, Detroit, Michigan, has kindly placed the following very important unpublished chemical studies with *L. acidophilus* at our disposal:

> 1. The total volatile acids approximate 5 to 10 per cent of the combined volatile and non-volatile acids. The different strains are quite uniform in this respect. Rettger's human strain and rat strain were studied. Kopeloff's human strain was also studied.

2. The non-volatile acid amounts to about 0.5 to 1.2 per cent of the weight of the culture depending upon the strain investigated.

3. The non-volatile acid of *acidophilus* is found to be entirely dextro-lactic acid. No traces of succinic or other acid being present.

4. The volatile acids consist of formic, acetic, and propionic. The proportions appear to be about 50 per cent formic, and nearly equal amounts of acetic and propionic. The method of volatile acid analysis used was that of Gillespie and Walters.

5. A very thorough growth study of *acidophilus* showed that in a pure hydrogen or nitrogen atmosphere (free from molecular oxygen as far as possible) it grew less rapidly than it did in an atmosphere of pure oxygen. However, the cultures reached their limiting pH in either atmosphere (about 3.8 to 4.0 pH). The culture in pure oxygen soon reached their natural reduction state beneath the surface of the liquid (0.49 volts uncorrected for calomel potential).

According to Orla-Jenson, and others, *Bulgaricus* produces up to 1.7 per cent laevo-lactic acid. Currie found that many of the lactobacilli produced dextro-lactic acid.

I, personally, believe that one point of differentiation between *acidophilus* and *Bulgaricus* lies in the stereomorphism of the lactic acid, and I have found a culture of *Bulgaricus* that produces laevo-lactic acid according to careful analysis of the zinc salt. The *Bulgaricus* strain came from the St. Louis Dairy Laboratory. This is in agreement with other investigations on *Bulgaricus*.

The discussion of the literature on *L. acidophilus* has so far been confined to its occurrence and significance in the gastro-intestinal tract. Its presence in the mouth has yet to be considered. Howe and Hatch (85) have demonstrated that the "Moro-Tissier group of bacilli" is constantly present and predominant in the flora accompanying dental caries.[2] Rodriguez (153) claims that *L. acidophilus* is the etiological factor in dental caries. The same conclusion was arrived at independently and almost simultaneously by McIntosh, James, and Lazarus-Barlow (120) in England. However, the difficulties in establishing final proof are very great; for despite the fact that *L. acidophilus* may occur in the presence of dental caries, the actual production of the disease by this, and no other, organism is still required. To quote McIntosh and his co-workers (120) "experiments to produce caries artificially in rabbits have failed. There is slight evidence of success in the case of a monkey." Sierakowski and Zajdel (159) reported *L.*

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[2] The Research Laboratories of the Royal Dental Hospital, reported similar results in the *British Dental Journal 44*, 1923, 907.

acidophilus-odontolyticus in 80 per cent of cases with dental caries. Bunting and Palmerlee[3] in seventy-three cases found *L. acidophilus* in 100 per cent of initial caries, 94 per cent of advanced caries and in 16 per cent of mouths that were immune to caries. They concluded that there was an intimate association between *L. acidophilus* and the initial lesion of dental caries. Brilliant green and crystal violet, one per cent each in 50 per cent alcohol are recommended.

L. acidophilus has also been isolated from the human vagina. Jötten (91) claims that the "Doederlein Bacillus," an acid bacillus occurring in normal vaginal secretions, is identical with *L. acidophilus*. Naujoks (129) reported the isolation of *L. acidophilus* from the vaginal secretions of two-thirds of all cases of pregnancy and regarded it as identical with *B. vaginalis minor*.

In summarizing the literature on *L. acidophilus* which has been discussed, it becomes evident that the various investigators have been dealing with an organism which is recoverable from the gastro-intestinal tract, as opposed to the problematic occurrence of *L. bulgaricus*. Furthermore, the bulk of reliable evidence goes to prove that a simplification or transformation of the intestinal flora from a proteolytic to an aciduric type, dominated chiefly by *L. acidophilus* may be induced by the feeding of suitable carbohydrates or the ingestion of *L. acidophilus* organisms. Direct clinical observations, as well as indirect laboratory methods, indicate the practical importance of *L. acidophilus* and its therapeutic possibilities. Final proof will now follow.

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[3] Bunting, R. W., and Palmerlee, F. The rôle of *B. acidophilus* in dental caries. Proc. Soc. Exper. Biol. Med., *22*, 1925, 296–298.

CHAPTER III

CONTRIBUTIONS FROM YALE UNIVERSITY

The researches of Rettger and his associates bear much the same basic relation to *L. acidophilus* therapy as those which link the name of Metchnikoff and his colleagues with *L. bulgaricus.* If anything, the work of the former group represents a sounder development, more logical in its plan and more detailed in its scope, which culminates in certain definite clinical applications. However, it owes its inception to the stimulating theories and findings of Metchnikoff. In 1914, Rettger, Kirkpatrick and Jones (148) fed milk soured by *L. bulgaricus* to young chicks in an effort to combat bacillary diarrhea (142). No advantage in its use as compared with ordinary sour milk was observed. This investigation was further extended by Rettger, Kirkpatrick and Card (147) to include a study of rats.

> It was also shown that if large numbers of *B. bulgaricus* grown on the surface of ordinary nutrient agar were fed to rats which were on a bread and vegetable diet alone the bacteria did not appear in the feces, but were apparently destroyed somewhere in the digestive tract. On the other hand, the feeding of sterile milk or of lactose to rats subsisting on a bread and vegetable diet and in which the *acidophilus* type of bacteria was apparently absent, quickly brought about the development of numerous organisms of this type.

No data, however, were here included.

Again, Rettger and Horton (146) published some observations showing that a simplification of the intestinal flora of rats could be obtained by special diet, *L. acidophilus* frequently gaining predominance. In continuing the same investigation, Hull and Rettger (87), claimed that *L. bulgaricus* could not become acclimatized to the intestine of the rat, but that if the carbohydrate portion of the diet was augmented sufficiently, *L. acidophilus* could become the preponderant organism. The latter phenomenon was also observed in four human beings who consumed lactose with the daily diet for varying periods of time. However, the data from a quantitative standpoint have not been presented in detail, and for adequate demonstration one must turn to the later work of Rettger and Cheplin.

In a series of preliminary notes (35), (36), (37), (143), the way was paved for the treatise (144) whose comprehensive treatment of the subject makes it a classic in its field. Following an excellent abstract of the literature the first half of this treatise is devoted to feeding and implantation experiments with white rats, while the latter portion is reserved for a similar investigation of human subjects. Since the data are presented in great detail, a survey makes it evident that the following conclusions are legitimately deduced:

> Lactose and dextrin when given in sufficient amounts bring about a marked transformation of the intestinal flora in which *B. acidophilus* assumes particular prominence and may even completely supplant all other types of bacteria. Maltose, glucose and sucrose, on the other hand, have very little or no tendency to induce such a change. . . . . The daily administration of two cubic centimeters of the *B. acidophilus* suspension (nephelometer five) without the addition of the carbohydrates also leads to a profound change in the character of the flora of the white rat, and an implantation of *B. acidophilus* to the extent of 90 to 99 per cent of the total flora is effected. The simple character of the new flora persists so long as one or another of the above diets or preparations is continued, but reverts gradually to the normal or usual mixed type within five or six days after a return to the basal diet.

In studying *L. bulgaricus,* three rats were fed 2 cc. and three rats were fed 5 cc. of the suspension. The conclusion reached was that

> *B. bulgaricus,* which is not an intestinal organism, is unable under any circumstance to establish itself in the alimentary canal of the albino rat. Even the daily administration of 5 cc. of the *B. bulgaricus* suspension exerts no influence on the bacterial population of the intestine.

In accounting for the influence of lactose and dextrin on the intestinal flora, it is stated that:

> There appears to be a definite relation between the rate of absorption or digestion in the alimentary canal of a utilizable carbohydrate and its tendency to effect a transformation. Only those carbohydrates which reach the cecum and colon unchanged have a transforming influence. The process of elimination of the ordinary mixed flora and the establishment of *B. acidophilus* apparently does not depend on any changes in the hydrogen ion concentration within the intestine.

The results with human subjects closely follow those noted in rats, namely, the transforming influence of cultures of *L. acidophilus*, lactose, or dextrin, singly or in combination; the failure to implant *L. bulgaricus* in man; the incomplete absorption of lactose from the intestine; and the absence of any correlation between the hydrogen ion concentration of the stools and the prevalence of *L. acidophilus*. The treatise is concluded by an account of the preparation of *L. acidophilus* milk, the laboratory procedures employed and a general discussion of the transformation of the intestinal flora.

It is difficult to criticize this work otherwise than favorably, yet there are one or two points that might be raised. In all of the feeding experiments under consideration the dose of culture has been stated in terms of cubic centimeters of a standardized suspension. Such a unit is no real measure of the actual numbers of bacilli present even though it ensures relatively the same dose. That Rettger and Cheplin are not unaware of this fact may be gleaned from their own statement that "after several preliminary trials, beginning with 5 cc., 2 cc. of the standardized suspension were finally chosen, as less than this amount was found to be inadequate for bringing about a profound change in the intestinal flora." In other words there appears to be a minimum number of viable *L. acidophilus* necessary to bring about a transformation of the intestinal flora either in the rat or man, but these investigators have not specified this highly important information. To be sure, this is an error of omission rather than commission. Yet this point is of importance as will be demonstrated later. A similar difficulty obtains where these workers express bacterial findings in terms of relative percentages. When no actual quantitative data are presented there is no way of determining whether the reduction of Gram-negative organisms and increase in numbers of Gram-positive organisms is real or only apparent. From a therapeutic standpoint this is of significance. One wishes that Rettger and Cheplin had summarized all their findings in order to show what individual variations had occurred, even though space did not permit the publication of all data. Finally, in the all-important attempts to implant *L. bulgaricus*, their conclusion that this organism cannot be recovered from the intestinal tract after ingestion, which controverts the claims of Metchnikoff

(119) and his followers, is based on the complete feeding records of only four human subjects and nine rats. One cannot help but feel that perhaps Metchnikoff's work has been rather lightly disposed of.

From the experimental studies here discussed, Rettger and Cheplin (145) went on to the therapeutic application of *L. acidophilus*. A series of "pathologic cases," including twenty cases of chronic diarrhea following an attack of bacillary dysentery, three cases of dermatitis (eczema), three cases of colitis; and two cases of sprue were treated with *L. acidophilus* milk in many instances reinforced by lactose (except where contraindicated). Selected abstracts of these cases are published together with the statement that "a full account of these studies will be published, however, at an early date." So far as can be determined this has not appeared within the past three years and until such time as it does, judgment as to the validity of the conclusions must be suspended. The same may be said of the clinical observations of Cheplin and Wiseman (38) on a group of eight subjects and Cheplin, Fulmer and Barney (33), on the favorable results obtained in ten cases. Cheplin, Post and Wiseman (34) have reported favorable treatment with *L. acidophilus* milk in nine cases of gastro-intestinal disorder. Good case abstracts are given but no complete quantitative laboratory data are included. In a recent communication, Cheplin (32) has given daily defecation records for a series of cases of chronic constipation, chronic diarrhea and mucous colitis. However, the period before treatment has not been charted, so that an evaluation of the benefits of treatment is denied the reader. Also, bacteriological data are reported for only two cases. Nevertheless, there is no gainsaying that these investigators have succeeded in establishing *L. acidophilus* as a therapeutic agent in certain intestinal disorders which are otherwise particularly difficult to deal with medically.

The most recent contribution to our knowledge of *L. acidophilus* and *L. bulgaricus* is that of Kulp and Rettger (110). This represents a comparative study of these closely allied organisms along strictly bacteriological and biochemical lines. Reference has already been made to the difficulties inherent in the differentiation of these lactobacilli; therefore such an investigation is of more than usual interest. A number of strains of *L. acidophilus* and *L. bulgaricus* from different sources were employed. A study of media resulted in the development of a casein or Klim digest media apparently superior to ordinary media. Galactose proved to be the most favorable carbohydrate and Hitchen's (82) agar was more suitable than broth. The morphological and cultural characteristics of both species were found to be similar. Likewise no striking differences were observed in viability, thermal death point or certain immunological and physiological properties. Nevertheless, the fermentation of maltose, sucrose and unheated levulose was considered an excellent criterion for differentiation, inasmuch as *L. acidophilus* fermented these sugars and *L. bulgaricus* did not. But this criterion is not an absolute one, for exceptions were noted. Similarly, the development of acidity in milk, while usually greater with strains of *L. bulgaricus* than *L. acidophilus*, proved sufficiently variable to prevent its being used as an adequate means of differentiating these organisms. The chief consideration, therefore, appears to be the fact that "*L. acidophilus* can and does live and develop in the intestine of man and animals whereas *L. bulgaricus* is unable to do so." However, there are instances on record where *L. bulgaricus* has been recovered from the intestinal tract of man and animals which are as authentic as the contrary claim. It is nevertheless true that these findings are rather uncommon, as are the exceptions to the fermentation of maltose, sucrose, and unheated levulose.

Kulp and Rettger conclude that

> The differences noted between these two types constitute evidence enough for classifying them as separate species. . . . . However, the authors suggest that the Gram-positive rods designated *L. bulgaricus* and *L. acidophilus* be placed in the same species, with *L. acidophilus* as the central type. *L. bulgaricus* may be considered a variant of this type, variation being due to long culturing in milk. The chief differential characteristic of this variant is the fact that it has lost its ability to develop in the intestine. The central type, *L. acidophilus*, is a normal intestinal form which can continue to develop in the intestine, even when supplied as a milk culture.

Summarizing the contributions from Yale University to our knowledge of *L. acidophilus* and *L. bulgaricus*, there is no question but that they represent the most important body of information in the field. Comprehensive experiments with animals and man led early to the conviction that *L. bulgaricus* could not be implanted in the intestinal tract and that *L. acidophilus* could become so acclimatized. There followed the generally successful therapeutic application of *L. acidophilus* to intestinal disorders which had always proven refractory to medical treatment. Finally, bacteriological and chemical studies have yielded valuable data on the properties of *L. acidophilus* and *L. bulgaricus*.

CHAPTER IV

L. ACIDOPHILUS VERSUS L. BULGARICUS MILK FEEDING

From a survey of the literature on *L. acidophilus* and *L. bulgaricus* it becomes evident that the supporters of each are irreconcilable. The best evidence, that of Rahe (139) and Rettger and Cheplin (144), gives strong indication that *L. bulgaricus* cannot live in the human intestine while *L. acidophilus* can actually become so acclimatized. However, it should be remembered that the data of Rahe (139) as well as that of Rettger and Cheplin (144) are confined to experiments on only four human subjects. In view of the paramount importance of this point, which is really the crux of the whole problem, it appeared desirable to repeat such work on a larger series of cases.

The present study grew out of an investigation which the writer carried on during 1923–1924 at the Pasteur Institute in Paris in the Department of Physiological Chemistry under Professor Gabriel Bertrand and this opportunity is taken to express his deep appreciation of the courtesies extended to him. Data obtained there, using rats, in the main corroborated the findings of Rahe (139), to which reference has been previously made. However, they were equivocal enough to require further investigation, particularly as regards the implantation of *L. bulgaricus* in the human intestine. Consequently, the following procedure was employed.

Two or more fecal examinations were made on about 35 women (psychotic) to select a group in which no colonies showing the typical morphology of *L. acidophilus* or *L. bulgaricus* were found on whey agar plates. Seven constipated and five non-constipated subjects were thus selected for administration of milk fermented by *L. bulgaricus.* The organisms used for this purpose were: A strain called the Pasteur Institute Bacillus corresponding to Metchnikoff's original culture, kindly furnished by Dr. Legros of the Pasteur Institute, Paris; and two strains used by Kulp and Rettger, labelled B-3 and B-4 respectively, which Dr. Kulp of Yale University was kind enough to furnish. Cultures used for this study were propagated from a single bacterial cell. Dr. Morton Charles Kahn of the Cornell University Medical College very generously performed this painstaking technic. The method of preparing milk, plating, etc., was the same as described by us in employing *L. acidophilus* milk, the counts averaged about 200,000,000 viable organisms per cubic centimeter.

The twelve subjects were arranged in groups of four; two constipated, and two non-constipated, to receive milk fermented by the same strain of *L. bulgaricus.* (An exception to this plan was the substitution of a constipated subject, case 11, for a non-constipated one.) In order to conclusively establish the normal flora of these patients, three fecal examinations were made during a preliminary observation period of two weeks. Careful record was made of the number of bowel movements during this time, as well as the volume, color, and consistency of the feces. Where the movement was induced by glycerine suppositories, enemas, cathartics, etc., this was noted. Following the preliminary period of observation the subjects received one litre of *L. bulgaricus* milk daily in addition to the regular hospital diet. This administration of milk occurred for 14 days, during which time three fecal examinations were made. Duplicate plate counts were made from the dilutions 1:100,000 and 1:1,000,000. Cases 2, 8, 9, and 12 received the Pasteur Institute Bacillus; cases 4, 5, 7, and 10 received B-3, while cases 1, 3, 6, and 11 received B-4.

Whenever colonies were found on the plates which in any way resembled *L. bulgaricus,* several were fished and transferred to milk. Following this, if coagulation of milk was obtained, fermentation tests in triplicate were employed. Triplicate control tests on all three strains of *L. bulgaricus* used, showed rapid coagulation of milk and non-fermentation of maltose and sucrose, (although occasionally only after 5 or 6 days the sucrose tubes showed a very slight acidity). In only one instance, case 7, as will be seen in table 1, were any organisms found which fulfilled the physiological requirements of the organism ingested. Even here the morphology could scarcely be called typical of *L. bulgaricus.* Nevertheless, in an experiment of this nature, it is more desirable to give the benefit of the doubt to any disharmonious findings, rather than expect uniform agreement. Seventy-five per cent of the colonies on the plates from this subject were of the kind described. It is of interest in this connection to note that 2 days after treatment was discontinued another fecal examination failed to reveal the presence of the organism in question.

Considering the entire group of twelve subjects, therefore, it is evident that *L. bulgaricus* is not implanted in the human intestinal tract, although it may upon occasion be recovered from the feces. It should be mentioned in this connection that the study just described is a repetition of an earlier experiment dealing with the same subjects in the same way, except that the number of viable organisms per cubic centimeter was about 50,000,000 instead of 200,000,000.[1] The results showed that in the subject noted above colonies resembling, but not typical of, *L. bulgaricus* were recovered from the feces to the extent of 80 per cent of the total count. However, 5 days after treatment these had disappeared entirely. One other subject, case 12, showed 10 to 20 per cent of *L. bulgaricus*—like colonies during treatment and none subsequently. Since no fermentation tests were then employed and these results were not duplicated in the more carefully controlled repetition of this experiment, it is reasonable to believe that little significance need be attached to the earlier findings. The conclusion to be drawn from a consideration of this group of twelve subjects, therefore, is that *L. bulgaricus* is not implanted in the human intestinal tract, although occasionally it may be recovered from the feces after ingestion in large amount.

The possible influence of *L. bulgaricus* upon constipation may also be studied in table 1 where the data have been so arranged as to show the bowel condition of each subject, the number of days during which normal defecations occurred (third column) in the total number of days under observation which was 14. The third column represents the preliminary period of observation; the fourth column represents the period during which *L. bulgaricus* was administered. The fifth column gives the highest per cent of *L. bulgaricus*-like colonies found on the plates during the feeding period.

Since the period of observation and feeding were identical in point of time, namely 14 days, any differences in bowel condition due to *L. bulgaricus* will become evident by a comparison of the third and fourth columns. It will be seen that cases 1 and 5 remained unimproved. Cases 2, 6, and 8 showed slight improvement, while cases 3, 4, and 7 were still further benefited. The non-constipated cases continued in that condition. Before ascribing the above improvements to the beneficial influence of *L. bulgaricus,* it is of interest to inquire further into the condition of these patients upon the withdrawal of the milk. By consulting the sixth column it will be seen that cases 2, 3, and 5 returned to their original status, while cases 4, 6, and 8 regressed ever so

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[1] Thanks are due the *Journal of Laboratory and Clinical Medicine* for permission to reprint from *10*, 1925, 914–916.

TABLE 1

L. acidophilus versus L. bulgaricus milk feeding

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
CASE NUMBER	CONDITION	BEFORE TREATMENT (NUMBER OF NORMAL DEFECATIONS IN 14 DAYS)	DURING L. BULGARICUS FEEDING (NUMBER OF NORMAL DEFECATIONS IN 14 DAYS)	PER CENT L. BULGARICUS	INTERVAL, NO TREATMENT (NUMBER OF NORMAL DEFECATIONS IN 14 DAYS)	DURING L. ACIDOPHILIS FEEDING (NUMBER OF NORMAL DEFECATIONS IN 14 DAYS)	HIGH PER CENT L. ACIDOPHILIS	AFTER TREATMENT (NUMBER OF NORMAL DEFECATIONS IN 28 DAYS)
1	Severe constipation	2	1	0	1	10	84	12
2	Severe constipation	2	5	0	2	9	8	9
3	Constipation	3	10	0	5	12	100	22
4*	Constipation	5	11	0	7	3‡	78	
5	Constipation	7	8	0	7	14	99	26
6	Constipation	8	12	0	11	11	80	14
7	Constipation	4	8	75	10	11	72	14
8	Mild constipation	11	13	0	12	13	83	27
9	Non-constipation	14	14	0	14	14§	67	28††
10	Non-constipation	14	14	0	14	14§	99	28††
11	Non-constipation	14	14	0	14	14	84	28
12†	Non-constipation	14	14	0	14	1**	95	

* Deported.

† Paroled.

‡ In 6 days.

§ Two daily.

** In 1 day.

†† In 11 days.

slightly. The only clear cut improvement which persisted occurred in case 7. It is significant that this subject is the only one to show the presence of *L. bulgaricus*-like colonies in fecal platings. It should be remembered, however, that the benefits here indicated are not necessarily due to the presence of *L. bulgaricus* alone. As will be shown later in Chapter VII, the mere addition of one litre of milk per day to the regular diet may be expected to cause some increase in the number of defecations. Since the benefits from ingesting *L. bulgaricus* were not particularly striking, it is reasonable to believe that there is here little evidence in support of *L. bulgaricus* as a therapeutic agent in constipation.

Following an interval period of 14 days after the ingestion of *L. bulgaricus milk*, during which time three fecal examinations were made on all patients and no *L. bulgaricus*-like colonies found, the same subjects were fed *L. acidophilus* milk prepared in the same way and averaging about 200,000,000 viable *L. acidophilus* per cubic centimeter. The striking influence of this feeding on the number of daily defecations is noted in the seventh column of table 1.

It will be noted that the most severely constipated subjects, cases 1 and 2, yielded successfully and in approximately the same degree to treatment. Instead of about one normal defecation per week before treatment, these patients had frequent defecations, or 9 to 10 in 14 days, to be precise. Furthermore, a change in consistency from hard to soft feces was observed in cases 1, 3, 5, and 7. Again, improvement over the original condition may be noted in all the remaining constipated cases. Two of the non-constipated cases, 9 and 10, had two normal defecations every day during treatment where previously such an occurrence was infrequent. There is no question, therefore, that *L. acidophilus* has a prompt and beneficent influence on constipation. Comparing the results of *L. acidophilus* with *L. bulgaricus* milk feeding, it will be seen from table 1 that in every constipated case but one, and in two of the non-constipated cases, there have been a greater number of normal defecations during *L. acidophilus* milk treatment. Of even greater significance is the fact that relatively high percentages of *L. acidophilus* have been recovered from the feces of these patients during *L. acidophilus* feeding. The eighth column of table 1 brings this out clearly. Every subject, with one exception, had 67 per cent or more *L. acidophilus* colonies on the fecal plates, three having 99 to 100 per cent. Typical colonies were fished from all plates and fermentation tests indicated that they were identical with the *L. acidophilus* ingested. All cultures coagulated milk and fermented both maltose and sucrose. A majority also fermented unheated levulose.

The figures in the eighth column represent the highest per cent of *L. acidophilus* attained in three analyses. It will be remembered that when *L. bulgaricus* was fed for a similar period of time only one patient revealed its presence in the feces. The large per cent of *L. acidophilus* found is conclusive evidence that these two organisms differ markedly in their ability to remain alive in the human gastro-intestinal tract.

It is of interest to observe the bowel condition of the patients after treatment with *L. acidophilus* milk. The ninth column of table 1 carries such data. It will be seen that in the constipated group, with but one exception, there were a greater number of normal daily defecations than in the period before any treatment was begun. Two of the four non-constipated cases continued to have two normal defecations daily after, as during, *L. acidophilus* therapy, although after 11 days they regressed to a single normal defecation per day. The most striking improvements which persisted after discontinuing *L. acidophilus* milk are to be noted in cases 1, 3, 5, and 8. Obviously where the feeding period is as short as 14 days, one could scarcely expect the benefits to persist very long after discontinuing treatment. That some success obtains is encouraging from the standpoint of permanent benefit. As compared with the period during ingestion of *L. acidophilus* milk, the number of normal daily defecations after treatment is less in five of the eight constipated cases, the same in one, and more in another case.

Fecal analyses made during this period subsequent to treatment showed that *L. acidophilus* had disappeared in one week from all cases. After 2 weeks no *L. acidophilus* could be recovered from the feces of any patient. It is thus apparent that *L. acidophilus* has not persisted to any appreciable degree after treatment has been discontinued. However, this is not surprising since the period of feeding was of such short duration. It will be shown in another connection that *L. acidophilus* may be recovered from the feces long after ingestion, providing feeding was carried on for a considerable period of time.

Summarizing the results of *L. acidophilus* versus *L. bulgaricus* feeding in twelve cases, the following points have been established within the limitations of the experiment:

1. *L. bulgaricus* can rarely, if ever, be implanted in the human intestine. In only one instance out of twelve were *L. bulgaricus* organisms recovered in the feces after 2 weeks of feeding in large quantity.

2. *L. acidophilus* was recovered from the feces of all of the same patients ingesting this organism in large numbers. In all but one instance over 67 per cent *L. acidophilus* was found by plate count during ingestion; 95 to 100 per cent being found in four instances. Two weeks after treatment no *L. acidophilus* could be recovered, due to the short duration of feeding.

3. Constipation was markedly alleviated by administration of *L. acidophilus* milk containing about 200,000,000 viable organisms per cubic centimeter. In non-constipated cases there was a tendency to increase the number of normal defecations per day. The benefits of *L. acidophilus* therapy persisted after treatment and in all but one instance the condition after treatment was better than that which prevailed before treatment was instituted.

CHAPTER V

CLINICAL RESULTS

I. PRELIMINARY[1]

In the preceding chapter it has been shown that *L. acidophilus* actually accomplishes much that Metchnikoff expected of *L. bulgaricus*, which has failed to fulfil its promise. It is well known that patients in institutions commonly suffer from chronic constipation. The present investigation, therefore, was undertaken, in order to ascertain the benefits, if any, to be derived by psychotic patients from such treatment. In a sense this represents one aspect of the relation of focal infection to the functional psychoses (which has been reported elsewhere (106)). Some of the methods employed at the New Jersey State Hospital at Trenton, N. J., for the determination and treatment of focal infections have been subjected by us to critical analysis (107), and therefore the attempt was made to experiment with some measure which could be substituted for the major intestinal surgery there resorted to for the alleviation of chronic intestinal stasis.

Two groups of subjects were used, the first consisting of seven "controls," who were mentally normal, and the second group consisting of seven female patients classed as dementia praecox and manic-depressive. Whole milk was used in the present instance instead of skimmed milk, and the bacterial counts were made weekly by microscope with occasional platings on whey agar. The usual daily administration was 1000 cc. of *L. acidophilus* milk and 300 gram of lactose, half the amount being taken between dinner and supper, and the remainder between supper and bedtime. This amount, however, was reduced as considered advisable, particularly in the controls. In general, to obtain the most palatable product, the milk was incubated at 35°C. for twenty hours. A fresh "resting" culture of the strain, kindly furnished us by Dr. Leo F. Rettger, was used every 2 weeks.

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[1] Reprinted from Jour. Am. Med. Ass., *79*, August 19, 1922, 609–611.

The results obtained with treatment after about six weeks are shown in table 2, and are best discussed individually. Considering the first psychotic subject, case 13, it will be seen that for the 3-month period of observation preceding the administration of milk and lactose there were very few defecations without enemas. However, within three days after treatment was commenced a normal daily evacuation was obtained. In this instance the daily administration of lactose was reduced from 300 to 100 grams owing to complaints of fulness in the abdomen. Case 14 showed an equally satisfactory adjustment. Case 15 presents virtually the same course except that the evacuations do not occur daily with the same regularity. The relief, however, from constipation in these cases is particularly noteworthy in view of the fact that their mode of life was unchanged.

TABLE 2

Therapeutic effect of L. acidophilus

CASE NUMBER	BEFORE TREATMENT		DURING TREATMENT	
	Number of normal defecations	Number of days	Number of normal defecations	Number of days
	Patients with psychoses			
13	17	93	42	53
14	1	25	6	6
15	7	37	28	49
16	11	103	13	28
17	24	66	7	9
18	49	92	6	48
19	95	116	26	49
	Non-psychotic subjects			
20	Monthly diarrheal attack, interval constipation		No diarrhea in 6 months; daily normal defecation	
21	Sporadic diarrhea tri-monthly		One diarrheal attack in 12 months	
22	11	30	61	96
23	16	30	43	43
24	10	30	46	46
25	0	30	0	9
26	23	30	30	30

The diet remained the same, except for the fact that somewhat less was eaten at meal time. It may be mentioned that the hospital diet is not high in animal protein. Case 16 presents a similar picture, with perhaps more irregularity in bowel movement. Case 17 showed the most immediate response, even to the extent of becoming diarrheal. The omission of lactose soon resulted in a more normal condition. Cases 16 and 17 became so agitated as to make continuation of treatment impracticable. Case 18 showed little change in condition, having about one normal defecation a week, despite the increase to 1500 cc. of milk administered daily. This patient took almost no other food, which may in part account for the difficulty. Furthermore, there appeared to be considerable voluntary inhibition to defecation at all times. She has since obtained a normal daily defecation. Case 19 had frequent bowel movements before treatment, but these were not of a very satisfactory nature. The addition of only 250 cc. of *L. acidophilus* milk increased the daily amount of feces and reduced their putrefactive character.

Turning now to the mentally normal subjects, the first two, cases 20 and 21, had had, previous to treatment, severe diarrheal attacks; the former suffered these at the rate of about one a month, with an intervening period of constipation, while the latter was a prey to less frequent sporadic attacks, approximately trimonthly. For the 6 months during which case 20 took 1000 cc. of *L. acidophilus* milk and 100 grams of lactose daily, the patient experienced no diarrheal attack during this period, but had one 3 weeks after discontinuing treatment and still another 3 weeks later. Case 21 had no diarrheal attack for 11½ months while taking 700 cc. of *L. acidophilus* milk, but then had a mild attack after treatment was discontinued. Case 20 had a normal daily defecation (sometimes two), and this despite a rather high animal protein diet;[2] and case 21 had a daily movement which was usually not satisfactory in size. In the latter case it was not considered advisable to increase the amount of milk administered or to reinforce it with lactose, because this patient did not wish to gain weight. The next subject, case 22, might be considered as a mild case of constipation, i.e., necessitating some form of treatment such as hot water or a weekly laxative. She took only 500 cc. of *L. acidophilus* milk daily; and, while the results were not striking, nevertheless a certain definite beneficial effect could be recorded. Case 23 was interesting, as the use of laxatives was required about twice every week. Any ingestion of *L. acidophilus* milk stimulated an immediate response, and the chief difficulty was to find a minimal amount, since the milk acted as a purgative. Case 24 had to use cathartics every other night. The administration of *L. acidophilus* milk and lactose caused satisfactory daily defecations. Case 25, who had undergone rectal operations, took 1000 cc. of *L. acidophilus* milk and some lactose, but obtained no beneficial results in 9 days. Three enemas and a cathartic were taken during this period. The patient preferred to discontinue the treatment. Case 26 had had normal daily defecations, but on taking about 300 cc. of *L. acidophilus* milk daily, the color and consistency of the feces were much improved.

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[2] Since discontinuing treatment, this subject has had two severe diarrheal attacks within 6 weeks.

TABLE 3

Relative percentage of Gram-positive and Gram-negative organisms in feces (weekly samples)

CASE NUMBER	3/24*		3/27*		4/3		4/11		4/18		4/25		4/29		5/2		5/9	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
13	33	67	21	79	21	79	68	32	46	54	50	50	39	61	36	64	40	60
15	30	70	16	84	25	75	47	53	41	59	70	30			25	75	24	76
16	19	81	15	85	16	84	65	35	66	34	25	75†	42	58	36	64	78	22
17	36	64	14	86	16	84	20	80†										
18	37	63	24	76	18	82	40	60	40	60	44	56	38	62	38	62	30	70
19															58	42	64	36
20			74	26			63	37										
21			49	51	28	72					38	62						
22			62	38														

* Before treatment.

† Milk discontinued.

In general, then, it may be said that the subjects receiving *L. acidophilus* milk and lactose obtained relief from constipation and diarrhea. It is further interesting to note in this connection that even after discontinuing treatment, case 16 continued to have a daily defecation.

Considering now the changes in intestinal flora induced by the ingestion of *L. acidophilus* and lactose, the relative percentages of Gram-positive and Gram-negative organisms as present in the feces and examined weekly are shown in table 3.

While the results are not very striking, nevertheless, it will be seen that in general there is a tendency toward an increase in the relative proportion of Gram-positive organisms. The failure to obtain a greater ascendency of *L. acidophilus* in the intestinal flora of these patients may be due to the fact that the pabulum has been *whole* milk and not skimmed milk as employed by Rettger and Cheplin (144). Obviously, the Gram-negative bacteria have a more suitable opportunity for development in this medium.[3]

An interesting feature of this treatment is the accompanying gain in weight, in two psychotic patients and four controls, of about 3 to 5 pounds. While it might be expected that the daily ingestion of milk would bring about such results, nevertheless, in a majority of instances, there has been a corresponding reduction in food intake, which possibly argues a somewhat enhanced absorption of nutrients. The change in the character of the feces is also of considerable importance. Almost invariably the actual amount has increased, while the color has become considerably lighter, and the consistency softer. With the carmin test, before treatment, no carmin could be detected in the feces of three of the patients after 48 hours even with an enema; and three patients showed carmin from 48 to 72 hours after ingestion. After treatment the carmin was expelled in approximately one-half the time required before treatment.

Within the limitations of the data under consideration, it is evident that with the ingestion of *L. acidophilus* there is a change in the intestinal flora, as shown by Rettger and Cheplin (144), and relief from constipation and diarrhea. In other words, instead of major surgery for the alleviation of a condition concomitant with chronic constipation, *L. acidophilus* treatment might well be worth a trial. Next in importance to the actual relief from chronic constipation is its influence on the psychoses. So far, within this limited period of time, this appears to be negative.

Cases 13 and 14, each classified as a case of dementia praecox, evidenced no change in their mental reactions during the period of treatment.

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[3] By using skimmed milk we have since obtained fecal plates containing 100 per cent *L. acidophilus* colonies from a psychotic patient.

Case 15, who had a recurrent depression, became somewhat brighter, less depressed and more communicative during the period of 6 weeks' treatment. However, she continued retarded in her activity as compared with her normal condition, and, on the whole the improvement was not striking.

Case 16, a case of dementia praecox, with ideas of influence and poisoning, resisted taking the milk practically during the whole period of treatment; this resistance became greater, attempts to feed the milk by tube were unsuccessful because of vomiting, and the treatment was discontinued after 6 weeks. During and following the treatment, there was less complaint about her bowels not moving, and she was relieved of the pain and distress which had occurred when enemas were given; but otherwise there was no change in the clinical picture. She remained idle, and retained her previous ideas.

Case 17 was at the time of the beginning of treatment showing a change from a depressed condition to a manic excitement. Her excitement during the week of treatment increased, she became entirely uncoöperative, and it was necessary to discontinue the milk.

Case 18 who had a recurrent depression, was mute and quite inactive in bed during the 7 weeks' period of treatment. Toward the end of this period she appeared to become slightly more alert, at times showed evidence of cheerfulness, and spoke occasionally. Otherwise, there was no improvement.

Case 19, who had had a recurrent manic excitement, was much improved at the time of beginning treatment, showing good self-control, and working industriously in the ward. It was not evident that, during the period of 2 weeks' treatment with *L. acidophilus* milk, there was any change in her clinical picture, except an increase in weight of 5 pounds.

During the foregoing investigation, certain points of interest arose, to which the following data refer. In the first place, it is of importance to determine the number of viable *L. acidophilus* organisms ingested daily. In table 4 is shown the daily analysis of milk both as regards viable *L. acidophilus* and acidity. It will be observed that the usual daily inoculums contained in the neighborhood of 100,000,000 to 200,000,000 viable *L. acidophilus* organisms per cubic centimeter. On the basis of 1000 cc. administered, this would amount to an ingestion of from 100,000,000,000 to 200,000,000,000 *L. acidophilus* organisms each day. The acidity of 10 cc. of the milk was neutralized by about 18 cc. of twentieth normal sodium hydroxid. Continued daily bacteriologic and clinical analyses over a period of about 6 weeks have approximated these figures.

TABLE 4

Daily analysis of L. acidophilus milk administered

DATE	AGE OF TRANSFER	INCUBATION OF MILK	ACIDITY, CUBIC CENTIMETERS N/20 NaOH FOR 10 CC. MILK	L. ACIDOPHILUS ORGANISMS, NUMBER PER CUBIC CENTIMETER
	days	*hours*		
4/19	5			240,000,000
4/20	6	22		240,000,000
4/21	7	21		190,000,000
4/22	8	19		260,000,000
4/24	10	20		430,000,000
4/25	11	20	24.1	200,000,000
4/26	12	21		300,000,000
4/27	13	21	21.8	130,000,000
4/28	14	23	20.2	135,000,000
4/29	15	19	16.3	210,000,000
4/30	16	17	15.4	22,000,000
5/ 1	17	22	18.4	Lost
5/ 2	1	23	5.4	3,500,000
5/ 3	2	22	16.8	27,000,000
5/ 4	3	21	10.7	30,000,000
5/ 5	4	24	13.1	90,000,000
5/ 6	5	19	12.8	1,500,000
5/ 7	6	18	17.1	145,000,000
5/ 8	7	22	15.9	64,000,000
5/ 9	8	24	23.4	70,000,000
5/10	9	25	18.2	75,000,000
5/11	10	22	16.4	140,000,000
5/12	11	24	14.5	135,000,000

In table 5 are shown some results in plating fecal suspensions on whey agar. The figures represent the averages of duplicate plates of the two highest dilutions. It will be seen that the relative proportion of Gram-positive to Gram-negative bacteria is even lower than shown by microscopic count. However, the last column is of immediate concern. The calculation of the percentage of ingested *L. acidophilus* organisms in the feces was made as follows: The subjects received 1000 cc. of milk containing approximately 200,000,000 *L. acidophilus* organisms per cubic centimeter, or a total of 200,000,000,000. A fair daily amount of feces would be about 100 gm. One hundred times the number of viable *L. acidophilus* organisms found per gram divided by 200,000,000,000 would yield the percentage of viable *L. acidophilus* organisms ingested, as excreted in the feces. The percentages recorded in the last column of table 5 are very small and might create some doubt as to whether any actual multiplication of *L. acidophilus* occurs in the intestine. Later, working with a series of cases in which the tranformation of the intestinal flora was more complete, twenty-six cases yielded an average of 6 per cent, the highest recorded being 25 per cent. Since the majority of cases thus examined show a relatively small amount of ingested *L. acidophilus* in their feces, it is difficult to believe that there is much multiplication of this organism in the intestine; it would seem that there is merely a survival.

TABLE 5

Gram-positive versus Gram-negative bacteria in fecal suspensions (plated on whey agar)

PATIENT	DATE	GRAM-POSITIVE, MILLIONS PER GRAM	GRAM-NEGATIVE MILLIONS PER GRAM	INGESTED L. ACIDOPHILUS ORGANISMS FOUND VIABLE IN FECES, PER CENT
13	4/29	18	40	0.90
15	4/25	5	13	0.25
16	4/28	9	39	0.45
18	4/28	1	31	0.05

In order to determine the keeping qualities of *L. acidophilus* milk, a series of three flasks was inoculated and incubated at 35°C. for twenty-four hours, and thereafter incubated for two weeks at room temperature (from 25° to 30°C.). They were analyzed daily for acidity and plated in duplicate as above for numbers of viable *L. acidophilus* organisms.

In table 6 are presented the averages for the three flasks, the last column indicating the probable error as calculated in the usual way. It will be seen that the numbers of viable organisms decline quite rapidly after three days, and then maintain a fairly low level. Also there was a marked rise in acidity after the first few days, and the milk consequently became less palatable. At the end of two weeks the taste was very unpleasant. This experiment was repeated twice again with similar results.

Subject to the limitations of the material under consideration, the following points have been established:

1. Relief from chronic constipation and diarrhea has been secured by the ingestion of *L. acidophilus* milk and lactose in mentally normal and psychotic subjects. This corroborates the work of Rettger and Cheplin (144).

TABLE 6

Average of daily analysis of L. acidophilus milk at room temperature

DATE	AGE OF TRANSFER	INCUBATION OF MILK	ACIDITY, CUBIC CENTIMETER N/20 NaOH FOR 10 CC. MILK	L. ACIDOPHILUS ORGANISMS, NUMBER PER CUBIC CENTIMETER, MILLIONS	PROBABLE ERROR
	days	*hours*			
4/27	1	30	22.4	177	± 2.4
4/28	2	25	18.8	184	±23.4
4/29	3	22	21.2	141	±17.4
4/30	4	23	22.9	44	±11.4
5/ 1	5	20	28.0	13	±11.4
5/ 2	6	20	29.6	30	± 3.6
5/ 3	7	24	34.9	12	± 1.2
5/ 4	8	23	33.0	9	± 1.4
5/ 5	9	25	36.9	19	± 1.8
5/ 6	10	25	38.2	20	± 4.2
5/ 7	11	21	39.0	25	± 0.7
5/ 8	12	23	40.0	$2\frac{1}{2}$	± 0.7

2. Five psychotic patients receiving such treatment showed no improvement in their mental conditions during the period of treatment; in two others improvement was slight, but was not more than might have been expected without treatment with *L. acidophilus* milk, each of these patients having recovered previously from similar attacks without this treatment.

3. The intestinal flora becomes changed on treatment with *L. acidophilus* whole milk and lactose, but the relative percentage of Gram-positive rods rarely exceeds 70 per cent.

4. Incubating *L. acidophilus* whole milk at room temperature is satisfactory for 3 or 4 days after which the number of viable organisms decreases rather rapidly, and the acidity increases to the point of unpalatability.

II. THE TREATMENT OF CONSTIPATION

Although it has been shown that *L. acidophilus* has been used successfully in the treatment of constipation and diarrhea, it would be difficult to state precisely how successful a therapeutic agent it is. Neither of the intestinal conditions dealt with—constipation and diarrhea—permit of exact definition; consequently, the interpretation of results must be somewhat arbitrary. For this reason, if for no other, it would seem desirable that investigators in this field present their facts as objectively as possible. Obviously, there are many factors which must be considered. The necessity for keeping a careful record of the frequency and character of defecations is well recognized. The dosage of *L. acidophilus* preparations is always of importance, but unfortunately it is unstandardized and therefore at present has little significance. Thus 500 cc. of *L. acidophilus* milk containing 200,000,000 viable organisms per cubic centimeter in the hands of one clinician is unquestionably more desirable than twice this amount or 1000 cc. containing only 50,000 viable organisms per cubic centimeter when administered by another. Since the transformation of the intestinal flora from the proteolytic to the aciduric type appears to depend on a mass inoculation, the importance of ingesting large numbers of viable *L. acidophilus* cannot be overemphasized. It is common experience that frequently in treating severe cases of chronic constipation it is necessary to increase the dose before obtaining beneficial results. We have given as much as 1500 cc. of *L. acidophilus* milk and 400 gm. of lactose daily in some instances before alleviating the constipated condition. Another practice that has proved valuable in difficult cases is the use of a high enema of *L. acidophilus* milk, thinned with a small amount of warm water. Generally these enemas are advised on two or three successive days. It is likely that in cases reported as failures, a sufficient increase in the number of viable organisms administered might have resulted in success. In this connection it may also be stated that the chief criticism against the commercial preparations of *L. acidophilus* now on the market is that the number of viable organisms they contain is too small to yield satisfactory results when compared with the mass inoculations made by scientific investigators. It is unfortunate, therefore, that the commercial products examined cannot bear recommendation.

It is not only important to study the influence of *L. acidophilus* on the intestinal condition as compared with the period immediately preceding treatment, but also to note how long any changes which have been induced persist. Previously these facts have been indicated, and the present report is concerned with the continuation of such an investigation. The female psychotic patients under treatment for constipation were twenty-seven in number, in addition to which there were also three mentally normal subjects. The *L. acidophilus* milk which was administered varied from 110,000,000 to 440,000,000 viable organisms per cubic centimeter, with an average of about 200,000,000 per cubic centimeter. Recent radiographic examination to determine intestinal motility in some of the patients caused them to be divided into two equal groups showing atonic and spastic constipation.

In table 7 are included all of the results of the treatment of constipation and diarrhea with *L. acidophilus* milk. It will be seen that this table has been so arranged as to show three periods: Before treatment, during treatment and after treatment. Under each period there are two columns, the second showing the number of days of observation, the first showing the number of days during which the normal defecations occurred. More than one normal defecation per day has been counted as only one defecation so as not to confuse the main consideration. The period after treatment has been divided into two parts: the first, to show the effect of continuing the ingestion of lactose alone; the second, comparable to the period before treatment.

The results speak for themselves. There remains no question as to the benefits to be derived from *L. acidophilus* therapy. While it is no panacea for all ailments, it is, nevertheless, an efficacious and simple therapeutic agent for the alleviation of chronic constipation. It is scarcely necessary to consider the data individually. Taking the cases in groups, cases 1 to 12 inclusive have already been discussed in detail in connection

TABLE 7

The influence of L. acidophilus milk on constipation

CASE NUMBER	BEFORE TREATMENT		L. ACIDOPHILUS MILK		L. ACIDOPHILUS MILK AND LACTOSE		AFTER TREATMENT			
							Lactose		Nothing	
	Number of normal defecations	Number of days	Number of normal defecations	Number of days	Number of normal defecations	Number of days	Number of normal defecations	Number of days	Number of normal defecations	Number of days
1	2	14	10	14					17	38
2	2	14	9	14					14	38
3	3	14	12	14					31	38
4	5	14	3	6						
5	7	14	14	14					35	38
6	8	14	10	14					21	38
7	4	14	11	14					14	28
8	11	14	13	14					27	28
9*	14	14	14‡	14					28†	28
10*	14	14	14‡	14					28†	28
11*	14	14	14	14					28	28
12*	14	14	1	1					28	28
13	17	93			42	53	10	10	473	608
14	1	25	6	6			17	23		
15	7	37			28	49	17	39		
16	11	103			13	28			282	440
17	24	66	7	9					2	2
18	49	92			71	141	46	126		
19	95	116	26	49	81	105	60	68	189	223
22	11	30	61	96						
23	16	30	43	43						
24	10	30	46							
25	0	30	0	9						
26	23	30	30	30						
27	2	14			46	62			25	41
28	15	48	36	49					42	62
29	0	36	106	149	44	60	9	13	103	170
30	9	34			46	70	52	65	64	76
31	1	7	22	63	131	216	99	154	23	71
32	0	14			151	241	61	63		
33	5	19			17	22			259	350
34	12	20			30	62			43	105
35	1	14			47	104				
36	1	11			30	52				
37	19	45	23	70	3	5			207	319
38	3	7	11	17					31	77
39	3	21			13	15	54	62	16	21

TABLE 7—*Continued*

CASE NUMBER	BEFORE TREATMENT		L. ACIDOPHILUS MILK		L. ACIDOPHILUS MILK AND LACTOSE		AFTER TREATMENT			
							Lactose		Nothing	
	Number of normal defecations	Number of days	Number of normal defecations	Number of days	Number of normal defecations	Number of days	Number of normal defecations	Number of days	Number of normal defecations	Number of days
40	1	7	7	17					25	109
41	3	7	60	70					191	251
42	3	15	58	83					145	175
43	2	10	40	83	90	188			5	25
44	1	27	39	62					60	68
45	0	28	0	142	0	50				
46	0	16	12	73	6	158				
47	0	6	7	7	125	167			29	53
48	0	11	7	38	20	77				
49	2	7	27	37	52	65				
50	7	21	16	40	69	192			24	41
51	14	21	28	40	27	32			101	171
52	5	16	41	56	26	32			68	127
53	7	21	23	40	28	32			126	135
54	10	17	23	40	83	191			121	240
55	5	30			53	56	66	66	924	924
56	4	30			152	166			43	64

* Non-constipated.

† Two normal defecations per day.

‡ Two normal defecations per day occasionally.

with table 2. The data in cases 13 to 19, however, have been amplified as compared with the presentation in table 2. It will be seen at a glance that the four most constipated cases, nos. 13 to 16, have been greatly benefited by *L. acidophilus* treatment. Discussion of the results after discontinuing treatment will be reserved for part III of this chapter. Cases 22 to 26 have, with the one exception already noted, shown marked improvement under treatment. Considering the remaining cases 27 to 56, the benefits derived during *L. acidophilus* treatment have been striking in a majority of instances. However, there were 5 cases, cases 34, 37, 45, 50, and 54, which showed very slight if any improvement. Circumstances did not permit of a careful radiographic examination of these patients to establish whether or not there was an obstruction or congenital anomaly. In several of these stubborn cases, in addition to the oral administration of *L. acidophilus* and lactose, enemas were given about twice a week of *L. acidophilus* milk thinned with warm water to permit flow. Despite these efforts, the patients remained as constipated as before treatment. It might be argued that the exception but proves the rule. Surely, conclusive evidence is not lacking as regards the efficacy of *L. acidophilus.* In a number of instances patients who previously averaged not more than one normal defecation per week had frequent normal defecations, that is to say, about two in three days, or every other day, as witness cases 1, 2, 29, 31, 32, 35, 36, 44, 47, 55, and 56.

The importance of lactose in *L. acidophilus* therapy is well recognized. It is, therefore, of interest to compare the feeding of *L. acidophilus* milk alone, *L. acidophilus* milk reinforced by lactose, and lactose alone after ingestion of *L. acidophilus* milk. One-third of the cases may be considered valuable in making such a comparison. In the first place, strange as it may appear, there does not seem to be much improvement when lactose is added to *L. acidophilus milk.* Thus cases 29, 43, 45, 47, 48, 49, 50, and 54 benefited very little, if any, by the addition of lactose. One case in this series, no. 31, did show decided improvement. It might be inferred that on the whole, lactose is of little value in addition to *L. acidophilus.* However, it will be noted that in all the instances cited, the period of time during which *L. acidophilus* milk was reinforced by lactose, was greater than the period of time during which *L. acidophilus* milk alone was consumed. This means that there is less experimental error in calculating the results of the former period. The use of lactose alone, after treatment with *L. acidophilus* milk, alone or with lactose, is valuable in prolonging the beneficial effects of *L. acidophilus,* as will be observed in all of the cases but one (case 18). This is further borne out by the fact that when lactose is discontinued the patient tends to have fewer normal defecations. While the number of cases is too small to validate any sweeping generalizations, nevertheless, it is evident that the beneficial effects of *L. acidophilus* treatment can be prolonged by the use of lactose, even after the ingestion of viable organisms has been stopped. The alternative explanations which might account for this are: first, that lactose furnishes a suitable medium for

Plate II. Four-Day-Old Whey Agar Plate of L. acidophilus Colonies (Actual Size)

the development of *L. acidophilus* in the intestine and perpetuates the transformation of the intestinal flora; or, second, that lactose alone is responsible for the relief of constipation. While it is generally accepted that lactose has laxative properties, its use has never been accompanied by such uniformly good results as those obtained with *L. acidophilus*. The fact already noted, namely, that lactose added to *L. acidophilus* milk causes no great improvement over the use of the latter alone, further confirms this contention.

Subject to the limitations of the data presented, the following points have been established.

1. *L. acidophilus* milk, with or without lactose, has been administered to fifty-six persons, resulting in a striking increase in the number of normal defecations in a majority of instances. Patients who previously averaged not more than one normal defecation per week, had normal defecations almost daily as a result of treatment.

2. Lactose proved to be valuable in prolonging the beneficial effects of *L. acidophilus* milk feeding after the administration of viable *L. acidophilus* organisms was discontinued.

III. THE PERMANENCE OF RESULTS OBTAINED BY L. ACIDOPHILUS THERAPY

One of the most important questions which arises in connection with the therapeutic value of *L. acidophilus* is whether the relief obtained from constipation and diarrhea is permanent or merely transitory. From the data presented in table 7, there is no doubt that the beneficial effects of *L. acidophilus* therapy are in evidence so long as the treatment is in progress. However, if attention be directed to the period following treatment, some interesting facts are disclosed. Of the thirty-four non-constipated cases where daily observations have been carefully recorded after discontinuing treatment, twenty-seven cases or 80 per cent continued to have a greater number of normal defecations: six cases or 18 per cent, had approximately the same number, and one case or 3 per cent, had fewer normal defecations than before treatment as may be seen in table 7. There is, therefore, good evidence for believing that in most instances the beneficial effects of *L. acidophilus* continue after treatment has been discontinued. To be sure, the use of lactose is valuable in this connection, for in a majority of instances stopping the lactose resulted in fewer normal defecations.

If the data in the last two columns of table 7 are examined in detail, it will be seen that the beneficial effects of *L. acidophilus* therapy have been found to persist for as long as 924 days, or about 2½ years (case 55). It is significant that prior to treatment this subject averaged only one normal defecation per week. Other instances of more than a year's duration may be found in cases 13 and 16; of about one year's duration in cases 33 and 37; and of 3 to 6 months' duration in cases 19, 29, 40, 41, 42, 52, and 53. In general the longer the period of treatment, the longer will be the duration of the beneficial effects. Nevertheless, there are several instances where the period of treatment has been relatively short, i.e., about 2 months or less, and the duration of benefit considerably longer than that time (as in cases 1, 2, 3, 5, 8, 13, 16, 37, 40, 41, 52, 53, and 55).

TABLE 8

Permanence of results obtained by L. acidophilus

CASE NUMBER	BEFORE TREATMENT			NUMBER OF DAYS AFTER TREATMENT DISCONTINUED	PERIOD AFTER TREATMENT		
	Number of normal defecations	Number of days	Per cent normal defecations		Number of normal defecations	Number of days	Per cent normal defecations
13	17	93	18	980	8	24	33
16	11	103	11	408	16	26	62
31	1	7	15	433	22	26	85
43	2	10	20	488	5	25	20
55	5	30	17	896	28	28	100

These data are encouraging for they indicate that the effects of *L. acidophilus* therapy are not transitory phenomena dependent solely upon the continued administration of *L. acidophilus* milk. Whether these benefits are due to better bowel habits which are induced as a result of treatment or whether they are directly dependent on the presence of *L. acidophilus* organisms in the intestinal tract, remains still to be established. The mere fact that the benefits do persist is one of the most potent arguments in favor of *L. acidophilus* therapy.

In order to throw still further light on this important point, careful daily records were again kept for about a month on such patients as were still in the Hospital long after treatment had been discontinued. These data are presented in table 8.

It will be seen that all but one of these five cases showed improvement over their original condition from about one to three years after discontinuing treatment. Calculating the per cent of normal defecations (using the number of days as a divisor and the number of normal defecations as a dividend), a comparison of the figures before and after treatment is illuminating.

To summarize: The benefits of *L. acidophilus* usually persist after treatment has been discontinued. Eighty per cent of thirty-four cases had more normal defecations after, than before, treatment. Such benefits persisted in one case during three years of observation and in others for upwards of a year.

IV. THE TREATMENT OF DIARRHEA BY L. ACIDOPHILUS MILK

Significant as are the benefits to be derived from the administration of *L. acidophilus* milk in constipation, results even more striking may frequently be observed in the treatment of diarrhea. We have had under observation a group of diarrheal cases in which cultures of the feces have proven negative for cholera, dysentery, tubercular and protozoal organisms. The generally recognized principles of treatment in vogue were employed with conspicuous lack of success. Sour milk preparations of various kinds gave no relief. Consequently, the administration of *L. acidophilus* milk was indicated. Following our usual custom, 1000 cc. of *L. acidophilus* milk was given daily by mouth. Lactose is contraindicated in diarrhea and was, therefore, omitted. In the more severe cases which did not respond very readily to treatment the oral administration of *L. acidophilus* milk was augmented by the rectal administration of *L. acidophilus* milk in the manner already described. The results of such treatment were gratifying and are presented in Table 9 where the data have been so arranged as to show the number of defecations each day before, and during treatment. These numbers have been separated by commas.

Of the eleven diarrheal cases, 57 to 67, all were benefited by treatment in that there were fewer defecations per day and the feces became more solid in consistency, as well as lighter in

TABLE 9

The influence of L. acidophilus milk on diarrhea

Case No. 57. Before treatment
3, 2, 4, 4, 8, 2, 3, 2, 8, 10, 4, 4, 6, 6, 6, 2, 2, 3, 1
1000 cc. *L. acidophilus* milk
5, 5, 6, 4, 3, 5, 4, 2, 4, 0, 3, 2, 2, 1, 5, 2, 3, 2
5, 4, 3, 3, 0, 4, 3, 3, 3, 3, 3, 1, 1, 3, 3, 2, 1, 1
Remarks: Feces became more solid. Gained weight.
Case No. 58. Before treatment
7, 8, 6, 5, 6, 5, 7, 6, 5
1000 cc. *L. acidophilus* milk
3, 3, 4, 1, 1, 4, 1, 3, 3, 3, 1, 0, 1, 1
1, 2, 1, 0, 1, 1, 1, 1, 2
Remarks: Feces became more solid: formed. Change in color from green to brown.
Case No. 59. Before treatment
9, 9, 10, 9, 7, 8, 8, 6, 7, 7
1000 cc. *L. acidophilus* milk
6, 5, 4, 5, 4, 5, 6, 4, 4, 2, 1, 3
2, 2, 1, 1, 2, 4, 1, 1, 2
Case No. 60. Before treatment
6, 5
1000 cc. *L. acidophilus* milk
6, 7, 7, 7, 6, 5, 6, 4, 6, 6, 7, 8, 6, 4, 3, 5
6, 8, 5, 2, 2, 1, 2, 2
Remarks: Feces became more solid.
Case No. 61. Before treatment
4, 3, 3, 8, 4, 5, 4, 3, 4, 5, 4, 4, 2
1000 cc. *L. acidophilus* milk
2, 1, 2, 2, 3, 2, 2, 2, 2, 1, 1
Remarks: Feces changed from green fluid to dark-brown semi-formed.
Case No. 62. Before treatment
3, 7, 7, 3, 3, 3, 4, 4, 3, 4, 5, 3
4, 5, 4, 3, 3, 5, 5, 4, 3
1000 cc. *L. acidophilus* milk
4, 3, 2, 3, 2, 4, 3, 2, 4, 4, 4, 4, 2, 5, 4
5, 3, 2, 2, 3, 1, 2, 3, 3, 1, 2, 1

TABLE 9—*Continued*

Case No. 63. Before treatment

1, 4, 3, 2, 2, 4, 3, 0, 5, 4, 5

1000 cc. *L. acidophilus* milk

3, 3, 6, 3, 2, 2, 1, 1, 2, 3, 6, 4, 3, 2, 4, 5
3, 3, 4, 3, 4, 3, 1, 3, 4, 2, 1, 3, 2, 3, 2, 2, 1, 2
2, 1, 0, 0, 0, 2, 2, 0, 0, 0, 0, 1, 3, 2, 2, 1, 1
3, 4, 1, 3, 2, 5, 1,

Remarks: Feces became more solid and darker colored.

Case No. 64. Before treatment. Diarrhea record incomplete

1000 cc. *L. acidophilus* milk

1, 2, 1, 2, 3, 1, 1, 2, 1, 0, 1, 2, 2, 2, 1, 1, 1, 2, 2
2, 2, 1, 1, 2, 2, 3, 2, 1, 2, 3, 2, 1, 2, 2, 2, 3, 2
3, 1, 3, 1, 2, 1, 4, 3, 3, 2, 3, 2, 2, 1

Remarks: Feces changed from yellowish-green and watery to brown, normal, solid.

Case No. 65. Before treatment

2, 6, 3, 3, 2, 3, 4

1000 cc. *L. acidophilus* milk

3, 2, 4, 2, 4, 2, 3, 3, 2, 3, 2

Remarks: Feces changed from green fluid to occasional brown semi-solid.

Case No. 66. Before treatment. Diarrhea—data incomplete

1000 cc. *L. acidophilus* milk

2, 1, 2, 2, 3, 2, 4, 2, 3, 2, 3, 1, 2, 1, 2, 1, 2, 2
3, 2, 1, 1, 1, 2, 2, 1, 1, 3, 2, 3, 2, 2, 1, 3, 1, 1
4, 1, 1, 1, 3, 3, 2, 1, 2, 4, 4, 2, 4, 0

Remarks: Feces changed from green fluid to light-brown semi-solid.

Case No. 67. Before treatment

5, 6, 5, 4, 6, 5, 4, 5, 5, 4, 5, 4, 6, 5, 5, 4, 6
5, 4, 5, 5, 6, 5, 5, 5, 6, 5

1000 cc. *L. acidophilus* milk

2, 3, 2, 3, 3, 2, 3, 2, 3, 2, 2, 1, 2, 1, 2, 3, 3, 2
2, 2, 2, 1, 2, 2, 2, 2, 2, 2, 2, 2, 1, 2, 2, 2, 2, 1, 1

color. As might be expected in severe diarrheas, most of the patients lost appetite and weight. Although accurate weight records were not obtained, it was observed that most of these patients gained in weight under treatment. This was accompanied by better appetite. It would be of interest to have such patients under observation for a considerable period of time to note whether diarrhea might recur, but this was not practicable. The only evidence at our disposal concerns the two cases of sporadic diarrhea, cases 20 and 21, mentioned in table 2. In both these cases diarrheal attacks recurred after treatment had been discontinued. Since these two cases were not of a type similar to those discussed above, no inferences could legitimately be made.

The results of the treatment of diarrhea by *L. acidophilus* milk may be briefly summarized as follows:

Eleven cases of severe diarrhea, not of infectious type and two cases of sporadic diarrhea were successfully treated by oral administration of *L. acidophilus* milk, augmented by rectal administration when necessary.

To recapitulate the clinical results of *L. acidophilus* therapy discussed in the present chapter, it has been established within the limitations of the data under consideration that:

1. Relief from constipation has been secured in a series of fifty-two cases by the oral (and rectal) administration of 1000 cc. of *L. acidophilus* milk, with or without lactose. Of four non-constipated cases fed *L. acidophilus* milk for 2 weeks, two of these had an increased number of normal defecations daily.

2. Lactose proved to be valuable in prolonging the beneficial effects of *L. acidophilus* milk feeding after the administration of viable *L. acidophilus* organisms was discontinued.

3. The results obtained by *L. acidophilus* therapy appear to have a certain degree of permanence, even when the ingestion of lactose is stopped. Eighty per cent of the cases under observation after treatment was discontinued had a greater number of normal defecations than before *L. acidophilus* treatment was begun. One subject under observation for 3 years after *L. acidophilus* milk and lactose treatment was stopped, continued to have normal defecations daily. Prior to treatment, she averaged only one normal defecation per week. Others also

showed benefits upwards of a year after treatment was discontinued.

4. Eleven cases of severe diarrhea and two cases of sporadic diarrhea were successfully treated by the administration, oral and rectal, of *L. acidophilus* milk.

CHAPTER VI

THE INFLUENCE OF L. ACIDOPHILUS ON THE INTESTINAL FLORA

No discussion of *L. acidophilus* would be complete without a consideration of its influence on the intestinal flora. Reference has already been made to the fact that *L. acidophilus* tends to transform the human intestinal flora from one that is Gram-negative and proteolytic to a Gram-positive fermentative type. In tables 10 to 56 are presented in detail the data which should accompany the interpretation of the clinical results presented in Chapter V. These tables have been so arranged as to give chronologically the beginning and end of any course of treatment; the results of microscopic and plate counts; the amount, consistency and color of the feces; and the frequency of normal defecation. Under the column marked "Date" the number of the month is given first, followed by the day and the the year. Under "Treatment" is given the amount and kind of preparation used Under "Microscopic—*L. acidophilus*—per cent" is given the relative per cent of Gram-positive rods found upon microscopic examination of Gram-stained smears. Under "Plate—*L. acidophilus*—per cent" is given the relative per cent of *acidophilus*-like colonies found on the whey agar plates. Such percentages represent the average of each of two plates made from the two highest dilutions. The experimental error in the agreement of duplicate plates usually approximates 5 to 10 per cent. Under "Feces—Amount" is the amount, characterized as large, small, or moderate; under "Consistency" the consistency, characterized as hard, soft, or fluid; and under "Color," the color characterized as yellow, brown, or light brown.[1] In the last column "Number of normal defecations" is given the number of days during which normal defecations occurred since the previous date of observation. The number of defecations noted in the last column al-

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[1] It should be noted that the abbreviation "S" represents "small" in connection with amount of feces and "soft" in connection with consistency.

TABLE 10

Case 1

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/19/24	Nothing	32	0*	..	..	..	
12/24	“	22	0	S	H	B	1
1/2/25	1000 cc. *L. bulgaricus* milk	28	0	S	H	B	1
1/7	“	30	0	..	..	..	0
1/14	“	30	0	S	H	B	1
1/17	Nothing	25	0	..	..	..	0
1/19	“	28	0	..	..	..	0
1/22	“	27	0	..	..	..	0
1/26	“	12	0	S	H	B	1
2/2	“	24	0	S	H	B	1
2/6	1000 cc. *L. acidophilus* milk	..	..	S	H	B	1
2/9	“	32	57	S	H	B	2
2/16	“	16	71	M	H	B	5
2/19	“	..	..	M	S	B	3
2/20	Nothing	19	84	..	..	..	0
2/25	“	32	0	S	H	B	1
3/3	“	25	0	..	..	..	0
3/30	“	..	..	M	H	B	16

TABLE 11

Case 2

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/19/24	Nothing	30	0	..	..	..	..
12/24	“	..	..	S	H	B	1
1/2/25	1000 cc. *L. bulgaricus* milk	34	0	S	H	B	1
1/7	“	34	0	S	H	B	2
1/14	“	31	0	S	H	B	2
1/17	Nothing	24	0	S	H	B	1
1/19	“	23	0	..	..	..	0

S., small; M., moderate; L., large; H., hard; S., soft; F., fluid; Y., yellow; B., brown; L.B., light brown.

* By suppository.

TABLE 11—*Continued*

Case 2—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
1/22	Nothing	28	0	. .	. .	. .	0
1/26	“	40	0	S	H	B	1
2/2	“	56	0	. .	. .	. .	0
2/6	1000 cc. *L. acidophilus* milk	. .	. .	S	H	B	1
2/9	“	44	5	S	H	B	2
2/16	“	9	4	M	H	B	5
2/19	“	. .	. .	M	H	B	2
2/20	Nothing	27	8	. .	. .	. .	0
2/25	“	15	0	M	H	B	2
3/3	“	42	0	M	H	B	1
3/30	“	. .	. .	M	H	B	11

TABLE 12

Case 3

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/19/24	Nothing	30	0	. .	. .	. .	. .
12/24	“	41	0	M	S	LB	2
1/2/25	1000 cc. *L. bulgaricus* milk	16	0	M	S	B	1
1/7	“	28	0	M	H	B	3
1/14	“	18	0	M	S	B	5
1/17	Nothing	28	0	M	H	B	2
1/19	“	31	0	M	S	B	1
1/22	“	22	0	M	H	B	1
1/26	“	34	0	M	H	B	1
2/2	“	25	0	M	H	B	3
2/6	1000 cc. *L. acidophilus* milk	. .	. . .	M	H	B	2
2/9	“	25	100	M	S	B	2
2/16	“	23	99	M	S	B	7
2/19	“	. .	. . .	M	H	B	3
2/20	Nothing	67	81	. .	. .	. .	0
2/25	“	24	0	M	H	B	4
3/3	“	40	0	M	H	B	4
3/30	“	. .	. .	M	H	B	23

TABLE 13

Case 4

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/19/24	Nothing	33	0	. .	. .	. .	. .
12/24	“	36	0	M	H	B	2
1/2/25	1000 cc. *L. bulgaricus* milk	23	0	M	H	B	3
1/7	“	. .	. .	M	H	B	4
1/14	“	40	0	M	H	B	5
1/17	Nothing	43	0	M	H	B	2
1/19	“	33	0	. .	. .	. .	0
1/22	“	35	0	M	H	B	2
1/26	“	11	0	M	H	B	3
2/2	“	28	0	M	H	B	3
2/6	1000 cc. *L. acidophilus* milk	. .	. .	M	H	B	3
2/9	“	46	14	M	H	B	1
2/12	“	52	78	M	H	B	2

TABLE 14

Case 5

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/19/24	Nothing	34	0	. .	. .	. .	. .
12/24	“	24	0	M	H	B	3
1/2/25	1000 cc. *L. bulgaricus* milk	34	0	M	H	B	4
1/7	“	33	0	M	S	B	4
1/14	“	33	0	M	S	B	3
1/17	Nothing	24	0	M	S	B	1
1/22	“	24	0	M	H	B	1
1/26	“	27	0	M	H	B	2
2/2	“	30	0	M	H	B	5
2/6	1000 cc. *L. acidophilus* milk	. .	. .	M	H	B	3
2/9	“	40	45	M	H	B	3
2/16	“	21	58	M	H	B	7
2/19	“	. .	. .	M	H	B	3
2/20	Nothing	43	99	M	S	B	1
2/25	“	42	0	M	S	B	4
3/3	“	43	0	M	H	B	6
3/30	“	. .	. .	M	S	B	25

TABLE 15

Case 6

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/19/24	Nothing	49	0	..	..	..	..
12/24	"	37	0	M	H	B	2
1/2/25	1000 cc. *L. bulgaricus* milk	26	0	M	H	B	6
1/7	"	49	0	M	H	B	5
1/14	"	35	0	M	H	B	6
1/17	Nothing	35	0	M	H	B	2
1/19	"	40	0	M	H	B	2
1/22	"	31	0	M	H	B	2
1/26	"	23	0	M	S	B	3
2/2	"	20	0	M	H	B	6
2/6	1000 cc. *L. acidophilus* milk	..	..	M	H	B	3
2/9	"	61	80	M	S	B	2
2/16	"	41	0	M	H	B	7
2/19	"	..	..	M	H	B	1
2/20	Nothing	45	28	..	..	..	0
2/25	"	27	0	..	..	..	0
3/3	"	39	0	M	H	B	3
3/30	"	..	..	M	H	B	18

TABLE 16

Case 7

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/19/24	Nothing	34	0	..	..	..	..
12/24	"	26	0	M	H	B	2
1/2/25	1000 cc. *L. bulgaricus* milk	25	0	M	H	B	2
1/7	"	27	0	M	H	B	3
1/14	"	22	0	M	H	B	3
1/17	Nothing	19	0	M	H	B	2
1/19	"	34	0	M	H	B	2
1/22	"	16	0	M	H	B	2
1/26	"	35	0	M	H	B	2
2/2	"	31	0	M	H	B	6

TABLE 16—*Continued*

Case 7—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
2/6	1000 cc. *L. acidophilus* milk	..	..	M	H	B	2
2/9	“	34	72	M	H	B	3
2/16	“	29	6	M	H	B	6
2/19	“	..	..	M	S	B	2
2/20	Nothing	45	54	..	..	..	0
2/25	“	17	0	M	H	B	4
3/3	“	32	0	..	..	..	0
3/20	“	..	..	M	H	B	10

TABLE 17

Case 8

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/19/24	Nothing	26	0	..	..	..	..
12/24	“	30	0	M	H	B	5
1/2/25	1000 cc. *L. bulgaricus* milk	34	0	M	H	B	6
1/7	“	36	0	M	H	B	5
1/14	“	34	0	M	H	B	7
1/17	Nothing	20	0	M	H	B	3
1/19	“	13	0	M	H	B	1
1/22	“	16	0	M	S	B	3
1/26	“	21	0	M	H	B	3
2/2	“	20	0	M	H	B	6
2/6	1000 cc. *L. acidophilus* milk	..	..	M	H	B	2
2/9	“	36	83	M	H	B	3
2/16	“	16	32	M	H	B	7
2/19	“	..	..	M	H	B	3
2/20	Nothing	22	65	M	H	B	1
2/25	“	27	0	M	H	B	5
3/3	“	28	0	M	H	B	5
3/30	“	..	..	M	S	B	17

TABLE 18

Case 9

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/19/24	Nothing	30	0	..	..	...	..
12/24	"	32	0	M	S	LB	5
1/2/25	1000 cc. *L. bulgaricus* milk	24	0	M	S	B	8
1/7	"	25	0	M	S	B	5
1/14	"	29	0	M	S	LB	7
1/17	Nothing	15	0	M	S	LB	3*
1/19	"	40	0	M	S	Y	2
1/22	"	28	0	M	S	B	3*
1/26	"	25	0	M	S	LB	4
2/2	"	33	0	M	S	LB	6
2/6	1000 cc. *L. acidophilus* milk	..	..	M	S	LB	4*
2/9	"	38	9	M	S	LB	3*
2/16	"	34	50	M	S	LB	7*
2/19	"	..	..	M	S	LB	3*
2/20	Nothing	15	67	M	S	LB	1*
2/25	"	12	0	M	S	LB	5*
3/3	"	43	0	M	S	LB	6*
3/20	"	..	..	M	S	B	17*

TABLE 19

Case 10

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/18/24	Nothing	..	..	..	..	..	..
12/19	"	19	0	M	S	B	1
12/24	"	25	0	M	S	B	5
1/2/25	1000 cc. *L. bulgaricus* milk	23	0	M	S	B	8*
1/7	"	11	0	M	S	B	5
1/14	"	11	0	M	S	B	7*
1/17	Nothing	30	0	M	S	B	3
1/19	"	20	0	M	S	B	2
1/22	"	..	..	M	S	B	3

* Two normal defecations daily.

TABLE 19—*Continued*

Case 10—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
1/26	Nothing	16	0	M	S	B	4
2/2	“	22	0	M	S	B	6
2/6	1000 cc. *L. acidophilus* milk	..	..	M	S	B	4
2/9	“	13	60	M	S	B	3*
2/16	“	13	99	M	S	B	7*
2/19	“	..	..	M	S	B	3*
2/20	Nothing	30	72	M	S	B	1*
2/25	“	40	0	M	S	B	5*
3/3	“	36	0	M	S	B	6*
3/20	“	..	..	M	S	B	17

* Two normal defecations daily.

TABLE 20

Case 11

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/28/24	Nothing	..	..	..	..	..	..
12/19	“	34	0	M	H	B	1
12/24	“	30	0	M	H	B	5
1/2/25	1000 cc. *L. bulgaricus* milk	30	0	M	S	B	8
1/7	“	11	0	M	S	B	5
1/14	“	20	0	M	S	B	6
1/17	Nothing	40	0	M	S	B	3
1/19	“	31	0	M	S	B	2
1/22	“	26	0	M	S	B	2
1/26	“	27	0	M	S	B	4
2/2	“	21	0	M	S	B	6
2/6	1000 cc. *L. acidophilus* milk	..	..	M	H	B	3
2/9	“	60	73	M	S	B	3
2/16	“	12	83	M	S	B	7
2/19	“	..	..	M	S	B	3
2/20	Nothing	11	24	M	S	B	1
2/25	“	22	0	M	S	B	5
3/3	“	39	0	M	S	B	6
3/20	“	..	..	M	S	B	17

TABLE 21

Case 12

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
		per cent	*per cent*	Amount	Consistency	Color	
12/18/24	Nothing	..	..	..	..	..	..
12/19	“	18	0	M	H	B	1
12/24	“	42	0	M	H	B	5
1/2/25	1000 cc. *L. bulgaricus* milk	37	0	M	S	B	8
1/7	“	19	0	M	S	B	5
1/14	“	31	0	M	S	B	7
1/17	Nothing	20	0	M	S	B	3
1/19	“	41	0	M	S	B	2
1/22	“	18	0	M	H	B	3
1/26	“	21	0	M	S	B	4
2/2	“	41	0	M	S	B	7
2/6	1000 cc. *L. acidophilus* milk	..	..	M	H	B	4
2/7	Nothing	..	..	M	S	B	1

TABLE 22

Case 13

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
		per cent	*per cent*	Amount	Consistency	Color	
12/25/21	Nothing	..	..	..	..	...	..
3/28/22	1000 cc. *L. acidophilus* milk + 300 gram lactose	21	..	S	S	LB	17
4/3	“	21	..	S	S	Y	5
4/7	1000 cc. *L. acidophilus* + 100 gram lactose	..	..	S	S	Y	3
4/11	“	68	..	S	S	Y	4
4/18	“	46	..	S	S	Y	7
4/25	“	50	..	S	S	Y	7
5/2	“	36	..	S	S	Y	6
5/9	“	40	42	S	S	Y	7
5/16	“	35	38	S	S	Y	7
5/20	100 gram lactose*	..	..	M	S	Y	4
5/23	“	23	10	L	S	LB	3

* 1000 cc. *L. acidophilus* twice per week.

TABLE 22—*Continued*

Case 13—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
5/30	Nothing	19	5	M	S	Y	7
6/6	“	35	20	M	S	LB	7
6/13	“	45	3	M	S	Y	4
6/20	“	33	2	M	S	LB	7
6/27	“	23	0	S	S	LB	6
7/5	“	25	0	..	..	...	0
7/12	“	19	0	S	S	LB	2
8/29	“	..	..	S	S	LB	34
9/6	On parole—Nothing	..	..	..	..	...	...
1/7/23	“	..	..	S	S	B	94
1/10/24	“	..	..	S	H	B	311
2/18	Nothing	..	..	..	..	...	...
2/19	“	..	..	S	H	B	1
3/5	Transferred	..	..	S	H	B	7
3/11	“	..	..	..	..	...	...
3/12	Nothing	..	..	..	..	...	...
3/19	“	..	..	..	..	...	0

TABLE 23

Case 14

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
4/15/22	Nothing	..	..	S	H	B	1
5/2	“	20	..	..	..	..	0
5/10	1000 cc. *L. acidophilus* + 300 gram lactose	32	5	S*	H	B	0
5/16	200 gram lactose†	30	3	M	S	Y	6
5/24	“	39	5	M	S	Y	5
5/31	“	27	15	M	S	Y	7
6/7	“	..	..	M	S	LB	5

* By suppository.

† 500 cc. *L. acidophilus* twice per week.

TABLE 24

Case 15

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
2/19/22	Nothing			S	H	B	6
3/24/	“	30	..	S	H	LB	1
3/28	1000 cc. *L. acidophilus* milk + 300 grams lactose	16	..	..	..	..	0
4/3	“	25	..	L	F	Y	2
4/7	1000 cc. *L. acidophilus* milk + 100 grams lactose			L	F	Y	3
4/11	“	47	..	L	F	Y	0
4/18	“	41		L	S	YB	6
4/25	“	70	28	L	S	Y	4
4/29	“	..	..	L	S	Y	3
5/2	“	25	..	L	S	Y	2
5/9	“	24	1	L	S	Y	4
5/16	200 grams lactose*	13	31	L	S	Y	4
5/23	“	27	30	S	S	Y	3
5/31	“	2	1	L	S	Y	4
6/6	“	62	60	M	S	Y	2
6/13	“	65	0	L	S	Y	1
6/20	“ †	95	3	..	..	..	3
6/24	“ †	..	..	L	F	Y	4

* 1000 cc. *L. acidophilus* milk once per week.

† 1000 cc. *L. acidophilus* milk twice per week.

TABLE 25

Case 16

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/15/21	Nothing	..	..	..	..	..	
3/24/22	“	19	0	..	..	..	9
3/27	“	15	0	S	S	LB	1
3/28	1000 cc. *L. acidophilus* milk + 300 grams lactose	..	..	S	S	LB	1
4/3	“	16	..	S	S	Y	4
4/11	“	65	..	..	..	..	0
4/18	“	66	..	L	S	Y	4

TABLE 25—*Continued*

Case 16—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
4/25	Nothing	25	..	S	S	Y	5
5/2	“	36	..	M	S	LB	4
5/9	“	78	14	M	S	LB	5
5/16	“	23	8	M	S	LB	6
5/23	“	45	6	M	S	B	6
5/31	“	40	0	M	S	B	8
6/6	“	32	7	S	S	LB	4
6/13	“	44	3	M	S	LB	4
6/20	“	41	11	M	S	LB	6
6/27	“	41	2	M	S	LB	5
7/5	“	13	0	M	S	LB	7
7/12	“	28	0	M	S	LB	7
7/18	“	54	0	S	S	LB	5
7/25	“	19	0	M	S	LB	7
8/1	“	35	0	M	S	LB	5
8/8	“	17	0	M	S	LB	6
8/15	“	21	0	M	S	LB	4
8/22	“	38	0	M	S	LB	6
8/29	“	43	..	M	S	LB	4
9/5	“	31	..	M	S	B	3
9/12	“	28	..	S	H	B	5
9/19	“	26	..	M	S	B	7
9/26	“	34	0	M	S	B	7
10/3	“	46	64	M	S	B	7
10/10	“	54	0	M	S	B	7
10/18	“	36	25	M	H	B	7
10/25	“	48	0	M	S	LB	4
10/31	“	58	0	M	S	B	6
11/8	“	62	0	M	S	B	3
11/15	“	54	0	M	S	B	6
11/28	“	26	0	M	H	B	8
12/12	“	11	0	M	S	B	7
12/19	“	21	0	M	S	B	3
12/26	“	25	0	M	S	B	2
1/9/23	“	33	0	M	S	B	10
1/16	“	29	0	M	S	B	4
1/23	“	15	0	M	H	B	4
1/30	“	29	0	M	H	B	4

TABLE 25—*Concluded*

Case 16—Concluded

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
2/27	Nothing	23	0	M	S	B	15
3/16	“	33	0	L	S	B	7
3/23	“	26	0	M	S	B	4
3/30	“	21	0	M	S	B	1
4/6	“	16	0	L	H	B	2
4/13	“	23	0	M	H	B	5
4/27	“	40	0	M	H	B	4
5/4	“	23	0	L	H	B	3
5/11	“	20	10	M	S	B	6
5/18	“	28	0	M	S	B	3
5/25	“	28	0	M	S	B	4
5/31	“	62	0	M	S	B	3
6/13	“	84	0	M	S	B	6
6/21	“	28	0	M	S	B	7
7/8	“	21	0	M	S	B	9

TABLE 26

Case 17

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
1/20/22	Nothing	..	..	..	..	...	..
3/24/22	“	36	..	M†	S	LB	22
3/27	“	14	..	S	S	Y	1
3/28	1000 cc. *L. acidophilus* milk*	..	..	M	S	Y	1
4/3	“	16	..	L	S	Y	5
4/6	Nothing	20	..	L	S	Y	2

* 100 grams lactose caused diarrhea.

† By suppository.

TABLE 27

Case 18

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/26/21	Nothing	..	...	..	..	...	..
3/24/22	“	37	...	M	H	B	47
3/27	“	24	...	M	H	B	2
3/28	1000 cc. *L. acidophilus* milk + 100 grams lactose	..	...	..	..	...	0
4/3	1000 cc. *L. acidophilus* milk + 300 grams lactose	18	..	L	F	Y	1
4/11	1000 cc. *L. acidophilus* milk + 100 grams lactose	40	...	..	..	...	0
4/18	“	40	...	..	..	...	0
4/25	“	44	...	L	S	B	1
4/29	“	38	3	..	..	...	0
5/2	“	38	...	..	..	...	0
5/9	“	30	6	M	S	Y	2
5/16	“	16	8	S	S	Y	2
5/23	“	12	0	S	S	B	2
5/31	“	32	...	S	S	Y	4
6/6	“	29	67	S	S	Y	3
6/13	150 gram lactose*	73	12	M	S	Y	5
6/20	Nothing	65	98	S	S	Y	3
6/27	1000 cc. *L. acidophilus* milk + 300 grams lactose	66	92	S	S	B	1
7/5	“	41	100	S	S	Y	4
7/12	“	43	100	S	S	Y	4
7/18	“	58	100	S	S	Y	5
7/25	“	33	100	S	S	Y	6
8/1	“	52	50	M	S	LB	5
8/8	“	51	0	M	S	LB	6
8/15	300 grams lactose	34	0	S	S	Y	7
8/22	“ †	27	0	S	S	LB	7
8/29	“ †	27	0	S	S	LB	7
9/5	“ †	23	0	S	S	B	3
9/12	“	33	0	S	S	B	6
9/19	1000 cc. *L. acidophilus* milk + 300 grams lactose	27	0	M	H	B	3
9/26	“	40	50	S	S	B	7

* 1000 cc. *L. acidophilus* milk three times.

† 1000 cc. *L. acidophilus* milk twice.

TABLE 27—*Continued*

Case 18—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
10/3	300 grams lactose	18	0	S	S	B	7
10/10	“	60	20	M	S	B	6
10/18	“	56	85	S	S	LB	3
10/25	“	81	0	..	..	...	0
10/31	“	..	...	S	H	B	1
11/8	“	..	...	..	..	...	0
11/15	“	..	...	S	H	B	2
11/22	“	..	...	S	S	B	1
11/28	“	..	...	S	H	B	2
12/5	“	..	...	..	..	...	0
12/12	“	..	...	S	H	B	1
12/19	“	..	...	..	..	...	0

TABLE 28

Case 19

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/27/21	Nothing	..	..	..	..	...	..
4/21/22	500 cc. *L. acidophilus* milk	..	..	M	S	B	95
4/28	“	..	..	M	S	Y	7
5/2	“	58	..	M	S	Y	4
5/9	“	64	1	M	S	Y	6
5/12	200 grams lactose*	..	..	M	S	Y	3
5/16	“	39	9	M	S	Y	3
5/23	“	39	40	M	S	Y	7
6/1	“	28	60	M	S	Y	8
7/31	Operation	..	..	Incomplete			
8/1	Nothing	21	..	..	..	...	1
8/8	“	20	..	M	S	Y	7
8/15	“	24	..	M	S	Y	6
8/22	“	34	..	M	S	Y	7
8/29	“	32	..	M	S	LB	7

* 1000 cc. *L. acidophilus* twice per week.

TABLE 28—*Continued*

Case 19—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	per cent				
9/5	Nothing	34	..	M	S	LB	7
9/12	“	27	..	M	S	LB	7
9/19	“	26	..	M	S	LB	7
9/26	“	15	0	M	S	B	7
10/3	“	33	0	M	S	B	7
10/10	“	23	0	M	S	B	7
10/18	“	38	0	M	S	LB	8
10/25	“	21	0	M	S	B	7
10/31	“	28	70	M	S	B	6
11/8	“	40	25	M	S	B	8
11/15	“	37	..	M	S	B	7
11/22	“	33	2	M	S	B	7
11/28	“	30	5	M	S	B	6
12/12	“	17	0	M	S	B	14
12/13	Paroled	..	..	..	..	..	..
3/2/23	Nothing	25	0	Incomplete			
3/27	500 cc. *L. acidophilus* paste	..	..	S	H	B	1
4/2	Nothing	..	..	S	H	B	1
4/24	1000 cc. *L. acidophilus* milk	..	..	S	H	B	5
5/4	“	28	0	S	H	B	1
5/11	“	27	0	S	H	B	1
5/18	“	20	0	S	H	B	3
5/22	1000 cc. *L. acidophilus* milk + 300 grams lactose	..	..	..	..	..	0
5/25	“	40	0	S	H	B	1
6/28	“	8	0	M	H	B	13
7/6	“	28	0	M	H	B	8
7/20	“	31	5	M	H	B	13
8/2	1000 cc. *L. acidophilus* milk + 100 grams lactose	..	..	M	H	B	10
8/24	“	67	75	M	H	B	17
8/30	“	42	30	..	..	...	6
9/6	“	35	80	Incomplete			
9/13	“	85	70	“			
9/20	“	41	85	“			
9/27	“	43	0	..	..	...	7
10/2	100 grams lactose	39	15	..	..	...	6
10/18	“	36	1	..	..	...	14

TABLE 28—*Concluded*

Case 19—Concluded

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
10/25	100 grams lactose	37	80	..	..	...	7
11/9	“	58	50	..	..	...	13
11/16	“	17	0	..	..	...	7
11/19	Nothing	..	..	..	..	...	1
11/23	“	27	0	Incomplete			
11/30	“	16	0	M	S	Y	7
12/7	“	..	..	Incomplete			
12/14	“	..	..	..	..	...	7
12/28	“	..	..	..	..	...	10
1/4/24	“	..	..	..	..	...	6
1/11	“	..	..	..	..	...	5
1/25	“	..	..	..	..	...	11

TABLE 29

Case 27

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
8/18/22	Nothing	..	..	..	..	..	..
9/1	1000 cc. sterile milk	..	..	S	H	B	2
9/13	1000 cc. pasteurized *L. acidophilus* milk	16	0	M	H	B	2
9/19	1000 cc. *L. acidophilus* milk + 300 grams lactose	22	0	S	S	Y	3
10/3	“	47	0	S	S	B	5
10/10	“	34	1	M	S	B	7
10/18	“	51	5	M	H	LB	7
10/25	“	46	15	M	S	B	5
10/31	“	48	10	M	S	B	6
11/8	“	53	94	M	H	B	7
11/15	“	31	40	M	H	B	5
11/20	Nothing	44	1	M	S	B	4
11/28	“	11	0	M	S	LB	4
12/5	“	14	0	M	S	B	5

TABLE 29—*Continued*

Case 27—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/14	Nothing	21	4	M	S	B	7
12/20	“	..	..	Incomplete			
12/26	“	22	0	M	S	B	5
1/2/23	“	25	0	M	H	B	7
1/5	“	..	..	M	S	B	3

TABLE 30

Case 28

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
7/5/22	Nothing	9	0	Lost			0
7/12	“	10	0	..	..	..	0
7/18	“	33	0	..	..	..	0
7/25	“	23	7	..	..	..	1
8/1	“	20	0	..	..	..	5
8/8	“	25	0	..	..	..	5
8/15	“	34	0	..	..	..	2
8/22	1000 cc. *L. acidophilus* milk	27	0	..	..	..	2
8/29	“	35	0	..	..	..	7
9/5	“	20	0	..	..	..	3
9/12	“	35	0	..	..	..	4
9/19	“	30	0	..	..	..	5
9/26	“	13	0	..	..	..	6
10/3	“	29	0	..	..	..	5
10/10	Nothing	40	0	..	..	..	6
10/18	“	26	2	..	..	..	1
10/25	“	16	0	..	..	..	3
11/15	“	17	0	..	..	..	11
11/22	“	21	0	..	..	..	7
11/28	“	13	0	..	..	..	7
12/11	“	..	..	..	..	..	13

TABLE 31

Case 29

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
5/16/22	Nothing	..	..	..	..	..	..
5/31	“	28	..	..	..	..	0
6/6	“	33	..	..	..	..	0
6/13	“	36	0	..	..	..	0
6/19	1000 cc. sterile milk	39	0	..	..	..	0
6/27	1000 cc. *L. acidophilus* milk + 300 gm. lactose	51	0	M	H	B	0
7/12	“	44	0	S	S	Y	4
7/18	1000 cc. *L. acidophilus* milk	50	0	S	S	Y	2
8/15	“	17	0	S	S	Y	22
8/22	“	26	0	S	S	B	7
9/12	“	48	..	S	S	B	17
9/19	“	38	..	S	H	B	3
9/26	“	20	50	S	S	B	3
10/5	300 grams lactose	50	0	S	S	Y	6
10/10	“	63	0	S	S	B	3
10/18	Nothing	80	2	M	S	B	6
10/25	“	65	2	M	S	B	3
10/31	“	55	0	M	S	B	2
11/8	“	60	2	S	H	B	3
11/28	“	42	0	S	S	B	5
12/5	“	10	0	S	S	B	4
12/12	“	32	0	S	S	B	3
12/19	“	28	0	S	S	B	1
12/26	“	29	0	S	H	B	3
1/11/23	1000 cc. pasteurized *L. acidophilus* milk + lactic acid	25	0	S	H	B	0
2/9	1000 cc. *L. acidophilus* filtrate	31	0	S	H	B	3
3/3	1000 cc. *L. acidophilus* milk	42	55	S	H	B	2
4/6	“	40	0	S	H	B	13
4/20	“	36	0	M	H	B	13
5/22	1000 cc. *L. acidophilus* milk + 300 grams lactose	..	..	M	H	B	22
6/21	“	52	0	M	H	B	29
6/30	Nothing	..	..	M	H	B	9
7/20	“	23	5	M	S	B	19
8/2	“	33	0	M	S	B	13
8/16	“	37	0	M	S	B	13

TABLE 31—*Continued*

Case 29—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
8/24	Nothing	39	0	M	S	B	8
8/30	"	40	0	M	S	B	6
9/6	"	32	0	M	S	B	6
9/13	"	47	0	M	S	B	7
9/23	"	60	5	M	S	B	7

TABLE 32

Case 30

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
8/4/22	Nothing	..	..	..	..	...	..
9/6	1000 cc. sterile milk	20	0	S	H	B	9
9/13	1000 cc. *L. acidophilus* milk + 300 grams lactose	34	0	S	S	B	3
9/19	"	43	0	M	S	B	3
9/26	"	63	80	M	S	B	4
10/3	"	40	10	M	S	B	5
10/10	"	70	25	M	S	LB	6
10/18	"	65	0	M	S	LB	4
10/25	"	72	10	M	S	LB	5
10/31	"	74	40	M	S	LB	5
11/8	"	67	100	M	S	LB	6
11/15	"	46	85	S	S	LB	2
11/22	300 grams lactose	55	0	M	S	B	6
11/28	"	70	0	M	S	B	2
12/5	"	64	80	M	S	B	5
12/12	"	85	35	M	S	LB	7
12/19	"	85	15	M	S	B	7
12/26	"	85	40	M	S	B	6
1/2/23	"	80	10	M	S	B	4
1/9	"	85	0	M	S	B	7
1/23	"	85	0	M	S	B	12
1/26	Nothing	..	..	M	S	B	2

TABLE 32—*Continued*

Case 30—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
2 25	Nothing	..	..	S	S	B	24
3 3	"	..	..	Incomplete			
3 9	"	..	..	..	..	..	5
6 13	"	..	..	Incomplete			
7 1	"	..	..	M	S	B	14
7 23	"	..	..	M	S	B	21

TABLE 33

Case 31

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
7 18 22	Nothing	..	..	..	..	..	..
7 25	1000 cc. pasteurized *L. acidophilus* milk	19	0		Lost		1
8 1	1000 cc. *L. acidophilus* milk + 300 gm. lactose	26	0	..	..	..	2
8 8	"	33	25	..	..	..	6
8 15	"	51	..	..	..	..	5
8 22	"	27	..	..	..	..	6
8 29	"	60	..	..	..	..	7
9 5	300 grams lactose	22	..	..	..	..	7
9 12	"	43	..	..	..	..	7
9 19	"	12	..	..	..	..	7
9 26	"	19	0	..	..	..	7
10 3	"	25	0	..	..	..	6
10 10	"	62	0	..	..	..	3
10 18	"	54	0	..	..	..	4
10 25	"	37	0	..	..	..	6
10 31	"	48	8	..	..	..	6
11 8	"	56	0	..	..	..	5
11 15	"	17	0	..	..	..	1
11 22	"	52	0	..	..	..	7
11 28	"	36	50	..	..	..	5

TABLE 33—*Continued*

Case 31—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/5	300 grams lactose	18	0	..	..	..	7
12/12	"	33	1	..	..	..	6
12/19	"	45	0	..	..	..	6
1/9/23	"	40	0	..	..	..	11
1/16	"	62	0	..	..	..	1
1/23	"	45	0	..	..	..	1
1/30	"	56	5	..	..	..	3
2/6	Nothing	..	..	..	..	..	0
2/28	"	..	..	..	..	..	1
3/23	1000 cc. *L. acidophilus* milk	..	..	..	..	..	1
4/6	"	11	0	..	..	..	1
4/20	"	40	0	..	..	..	1
4/27	"	23	0	..	..	..	4
5/4	"	20	10	..	..	..	3
5/11	"	28	0	..	..	..	5
5/18	"	28	0	..	..	..	4
5/25	1000 cc. *L. acidophilus* milk + 300 grams lactose	17	10	..	..	..	4
5/31	"	44	30	..	..	..	5
6/30	1500 cc. *L. acidophilus* milk + 300 grams lactose	..	..	..	..	..	7
7/20	1500 cc. *L. acidophilus* milk + rectal *L. acidophilus*	85	15	..	..	..	3
8/2	"	33	0	..	..	..	2
8/16	"	85	40	..	..	..	5
8/24	"	85	10	..	..	..	4
8/30	"	85	5	..	..	..	3
9/4	"	49	0	..	..	..	3
9/13	"	38	0	..	..	..	
9/20	"	59	0	..	..	..	
9/25	"	48	0	..	..	..	
10/4	"	36	0	..	..	..	9
10/18	"	45	0	..	..	..	14
10/25	"	85	50	..	..	..	6
11/9	"	62	75	..	..	..	14
11/16	"	28	50	..	..	..	7
11/23	"	28	0	..	..	..	5
11/30	"	26	0	..	..	..	4

TABLE 33—*Concluded*

Case 31—Concluded

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
12/13	Nothing	..	..	..	..	..	9
2/19/24*	"	..	..	..	..	.	
2/20	"	..	..	S	H	B	1
3/10	"	..	..	S	H	B	14
3/12	"	..	..	Incomplete			
3/19	"	..	..	S	H	B	7

* 1000 cc. *L. acidophilus* milk once per week 9/19/22–1/30/23.

TABLE 34

Case 32

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
10/11/22	Nothing	..	..		Lost		0
10/25	1000 cc. pasteurized *L. acidophilus* milk	..	..	..	..	..	0
11/1	1000 cc. *L. acidophilus* milk + 300 grams lactose	..	..	..	..	..	0
11/22	1500 cc. *L. acidophilus* milk + 300 grams lactose	..	..	..	..	..	5
12/14	"	..	..	..	..	..	6
12/20	Operation—nothing	..	..	..	..	..	..
1/16/23	1500 cc. *L. acidophilus* milk + 300 grams lactose	44	5	..	..	..	21
1/23	"	85	65	..	..	..	4
1/30	"	47	25	..	..	..	3
2/5	"	24	0	..	..	..	5
2/13	"	21	0	..	..	..	7
2/21	"	30	50	..	..	..	4
2/27	"	59	0	..	..	..	3
3/8	"	30	2	..	..	..	8
3/16	"	26	15	..	..	..	6
3/23	"	9	1	..	..	..	5

TABLE 34—*Continued*

Case 32—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
4/13	1500 cc. *L. acidophilus* milk + 300 grams lactose	23	0	..	..	..	11
4/27	“	31	0	..	..	..	5
5/18	“	31	95	..	..	..	13
5/25	“	31	0	..	..	..	7
5/31	“	39	0	..	..	..	4
6/21	“	24	0	..	..	..	16
6/28	1000 cc. *L. acidophilus* milk + 100 grams lactose	18	0	..	..	..	6
7/6	“	26	40	..	..	..	8
7/20	“	28	75	..	..	..	13
8/2	100 grams lactose	27	0	..	..	..	12
8/10	“	24	0	..	..	..	3
8/24	“	27	0	..	..	..	18
8/30	“	31	0	..	..	..	6
9/6	“	34	0	..	..	..	7
9/13	“	30	5	..	..	..	
9/27	“	27	5	..	..	..	
10/25	“	41	10	..	..	..	27

TABLE 35

Case 33

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
8/18/22	Nothing	..	..	..	..	..	..
9/1	“	15	0	S	H	B	3
9/6	1000 cc. sterile milk	25	0	M	H	B	2
9/13	1000 cc. pasteurized *L. acidophilus* milk	30	0	S	H	B	2
9/19	1000 cc. *L. acidophilus* milk + 300 grams lactose	37	0	S	H	B	1
9/26	“	21	0	S	S	B	3
10/3	“	24	0	M	S	B	7
10/11	“	37	4	M	S	B	7
11/15	On parole—nothing	..	..	..	..	..	..
1/10/23	“	..	..	M	S	B	51
10/31	“	..	..	M	H	B	208

TABLE 36

Case 34

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
8/17/22	Nothing	..	..	..	..	..	..
8/29	"	15	0	S	H	B	8
9/6	1000 cc. sterile milk	25	0	S	H	B	4
9/13	1000 cc. pasteurized *L. acidophilus* milk	27	0	S	H	B	3
9/19	1000 cc. *L. acidophilus* milk + 300 grams lactose	29	0	S	H	B	2
9/26	"	61	5	M	H	B	4
10/3	"	34	60	S	S	B	2
10/10	"	70	45	S	H	B	5
10/18	"	63	80	S	H	B	3
10/25	1500 cc. *L. acidophilus* milk + 400 grams lactose	53	70	L	S	LB	1
10/31	"	28	75	M	S	B	6
11/8	"	52	25	M	S	B	3
11/16	1000 cc. *L. acidophilus* milk + 300 grams lactose	44	50	M	S	B	4
11/20	Nothing	36	30	M	S	B	2
12/5	"	33	80	M	S	B	7
12/12	"	85	40	M	S	B	5
1/2/23	"	22	1	M	S	B	9
1/16	"	38	25	M	S	B	5
1/23	"	18	80	M	S	B	2
1/30	"	73	75	M	S	B	1
2/5	"	31	0	M	S	B	3
2/13	"	30	25	M	S	B	3
3/4	"	..	..	M	S	B	8
12/3/24	"	..	1	Incomplete			
12/6	"	..	5	"			

ways represents the number of defecations occurring from the date on the preceding horizontal line to the day before the date on which the number occurs, inclusive. Thus, in table 10, for the 5 days following December 19, 1924, this patient was on the usual hospital diet with "Nothing" in the way of additional treatment. The data for these 5 days are given opposite

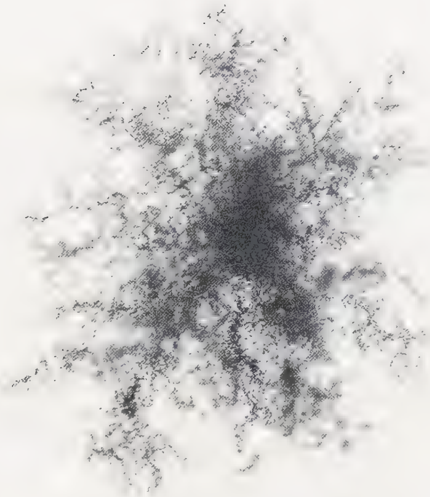

Plate III. Microphotograph of a Single L. acidophilus Colony on a Whey Agar Plate

Taken with Zeiss AA objective

TABLE 37

Case 35

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
10/12/22	Nothing	..	..	..	..	..	..
10/26	1000 cc. pasteurized *L. acidophilus* milk	..	..	M	H	B	1
11/3	1000 cc. *L. acidophilus* milk + 300 grams lactose	43	..	M	H	B	1
11/22	1500 cc. *L. acidophilus* milk + 400 grams lactose	..	..	M	H	B	4
11/28	"	54	95	S	H	B	1
12/14	Nothing	31		M	H	B	7
12/20	1500 cc. *L. acidophilus* milk + 400 grams lactose	..	..	Incomplete			
1/2/23	"	27	0	M	H	B	7
1/9	"	33	0	M	S	B	2
1/17	1000 cc. *L. acidophilus* milk + 300 grams lactose + rectal *L. acidophilus*	36	100	M	S	B	1
1/22	1500 cc. *L. acidophilus* milk + 400 grams lactose	85	90	M	H	B	3
1/30	"	85	100	M	H	B	6
2/5	"	37	0	M	H	B	5
2/13	"	37	99	M	H	B	8
2/19	"	..	..	M	H	B	7
2/20	Nothing	..	..	M	H	B	..
12/3/24	"	..	5	M	H	B	..
12/4	"	..	25	M	H	B	..

TABLE 38

Case 36

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
10/15/22	Nothing	..	..	..	..	..	..
10/25	1000 cc. pasteurized *L. acid*ophilus milk	..	..	S	H	B	1
11/1	1000 cc. *L. acidophilus* milk + 300 grams lactose	..	..	S	H	B	0

TABLE 38—*Continued*

Case 36—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
11/15	1000 cc. *L. acidophilus* milk + 300 grams lactose	42	0	S	H	B	5
11/22	1500 cc. *L. acidophilus* milk + 400 grams lactose	49	95	S	H	B	3
11/28	"	48	98	S	S	B	4
12/5	"	38	85	M	H	B	4
12/12	"	33	0	M	S	B	6
12/19	"	43	0	M	S	B	4
12/23	"	..	..	M	S	B	4

TABLE 39

Case 37

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
11/13/22	Nothing	..	..	..	..	..	..
12/30	"	..	..	M	H	B	9
1/5/23	1000 cc. pasteurized *L. acidophilus* milk	..	..	S	H	B	1
1/27	"	14	0	M	H	B	4
1/29	1000 cc. *L. acidophilus* filtrate	8	0	M	H	B	1
2/2	Nothing	..	..	M	H	B	3
2/18	1000 cc. *L. acidophilus* filtrate	33	0	S	H	B	2
3/6	"	..	..	M	H	B	11
3/13	1000 cc. *L. acidophilus* milk	..	..	M	H	B	4
4/6	"	16	0	M	H	B	6
4/13	"	18	0	M	H	B	2
5/4	"	38	25	M	H	B	5
5/11	"	29	35	M	H	B	1
5/18	"	30	50	M	H	B	3
5/26	1000 cc. *L. acidophilus* milk + 300 grams lactose	85	10	M	H	B	6
5/27	Nothing	..	..	M	H	B	3
6/10	On parole—nothing	..	..	..	..	..	9
12/16	On parole	..	..	M	S	B	115
5/26/24*	"	..	..	M	S	B	92

* Incomplete: 92 defecations in 129 days.

TABLE 40

Case 38

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
1/29/23	Nothing	..	..	..	..	..	..
2/5	1000 cc. *L. acidophilus* milk	16	0	..	..	..	3
2/13	"	28	100 (?)	..	..	..	6
2/22	Nothing	..	..	M	H	B	5
4/13	"	32	0	M	H	B	10*
4/20	"	47	0	M	H	B	6
4/27	"	24	10	M	H	B	7
5/5	"	13	0	M	H	B	8

* In 16 days.

TABLE 41

Case 39

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
6/24/22	Nothing	..	..	..	..	...	..
7/5	"	8	0	M	H	B	3
7/12	"	19	0	..	..	...	0
7/15	1000 cc. pasteurized *L. acidophilus* milk	28	11	M	S	LB	0
7/27	1000 cc. *L. acidophilus* milk + 300 grams lactose	70	0	S	S	Y	3
8/11	Operation—nothing	78	0	S	S	Y	13
8/15	300 grams lactose*	62	10	Incomplete			
8/22	"	51	..	S	S	B	5
8/29	"	32	..	M	S	B	6
9/5	"	46	..	M	S	LB	7
9/12	"	47	..	M	S	LB	6
9/19	"	44	..	M	S	LB	6
9/26	"	62	0	M	S	LB	6
10/3	"	37	10	M	S	LB	7
10/10	"	54	0	M	S	LB	7
10/16	"	..	..	M	S	LB	4
12/11	On parole—nothing	..	..	Incomplete			
12/31	"	..	..	M	S	LB	16

* 1000 cc. *L. acidophilus* milk twice per week.

TABLE 42

Case 40

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
2/14/23	Nothing	..	..	..	..	..	..
2/21	500 cc. *L. acidophilus* paste	40	0	..	..	..	1
3/8	“	28	0	S	H	B	1
3/13	1000 cc. *L. acidophilus* milk	..	..	S	H	B	5*
3/30	Nothing	33	0	S	H	B	7*
4/6	“	16	0	S	H	B	2
4/13	“	22	0	S	H	B	2
4/27	“	..	..	S	H	B	1
5/25	“	32	0	S	H	B	2
5/31	“	28	10	S	H	B	2
6/13	“	45	0	S	H	B	7
6/21	“	47	2	S	H	B	2
7/17	“	..	..	S	H	B	7

* Frequently refused milk.

TABLE 43

Case 41

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
2/14/23	Nothing	..	..	..	..	...	..
2/20	500 cc. *L. acidophilus* paste	..	..	M	H	B	3
3/8	“	25	0	M	H	B	7
3/13	1000 cc. *L. acidophilus* milk	32	95	M	H	B	2
3/23	“	13	100	M	S	LB	3
3/30	“	31	90	L	S	LB	7
4/6	“	41	100	M	S	LB	5
4/13	“	31	100	M	S	LB	7
4/20	“	54	80	M	S	LB	7
4/27	“	27	95	M	S	LB	7
5/4	“	28	85	M	S	LB	6
5/11	“	26	70	M	S	LB	7
5/18	“	49	95	M	S	LB	7
5/22	Nothing	45	0	M	S	LB	4

TABLE 43—*Continued*

Case 41—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
5/31	Nothing	30	50	M	S	LB	9
6/13	“	35	0	M	S	LB	13
6/21	“	42	0	M	S	LB	7
7/6	“	40	30	M	S	LB	14
7/20	“	27	0	M	S	LB	14
8/2	“	40	0	M	S	LB	12
8/16	“	35	0	M	S	LB	14
8/24	“	37	0	M	S	LB	6
8/30	“	32	0	M	S	LB	6
9/6	“	29	1	M	S	LB	7
9/13	“	38	90	M	S	LB	6
9/20	“	31	50	M	H	B	4
9/27	“	27	0	M	H	B	7
10/4	“	34	40	M	H	B	5
10/25	“	29	0	M	H	B	13
11/2	“	48	..	L	H	B	8
11/9	“	31	0	M	H	B	6
11/16	“	35	0	M	H	B	5
11/23	“	..	0	M	H	LB	5
11/30	“	..	0	Incomplete			
12/7	“	..	..	Incomplete			
12/14	“	..	..	M	H	B	2
12/21	“	..	..	M	H	B	1
12/28	“	..	..	M	H	B	2
1/4/24	“	..	..	M	H	B	4
1/11	“	..	..	M	H	B	6
1/18	“	..	..	S	H	B	4
1/25	“	..	..	M	H	B	3
2/4	“	..	..	M	H	B	4
2/11	“	..	..	Incomplete			
2/18	“	..	..	M	H	B	4

“12/24” when the fecal specimen was examined; and so on throughout this and the remaining tables in the series. On that day there were 22 per cent Gram-positive rods found upon microscopic examination. No *acidophilus*-like colonies appeared upon the whey agar plates. The feces were small in

TABLE 44

Case 42

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
2/14/23	Nothing	..	..	..	..	...	..
2/28	1000 cc. *L. acidophilus* milk	..	..	M	H	B	3
3/8	"	10	95	M	H	B	1
3/16	"	13	95	M	S	B	4
3/23	"	32	50	M	S	B	3
3/30	"	43	100	M	S	B	5
4/6	"	19	10	M	S	B	6
4/13	"	30	95	L	S	B	6
4/27	"	27	85	M	S	B	12
5/4	"	20	0	L	S	B	6
5/11	"	22	90	M	S	Y	5
5/18	"	30	75	M	S	LB	6
5/22	Nothing	31	0	M	S	LB	4
5/31	"	30	0	M	S	B	9
6/13	"	24	0	M	S	B	13
6/21	"	6	0	M	S	B	8
6/28	"	21	0	M	S	B	4
7/6	"	25	0	M	S	B	4
7/20	"	23	0	M	S	B	12
8/2	"	23	0	M	S	B	13
8/16	"	15	0	L	S	LB	14
8/30	"	37	0	L	S	B	12
9/6	"	28	0	M	S	B	5
9/13	"	..	50	M	S	B	5
10/25	"	11	0	M	S	B	31
11/9	"	33	0	M	S	B	14
11/16	"	11	0	M	S	B	4

amount, hard in consistency and brown in color. The patient had one normal defecation on only one day of the five under consideration. Any blank spaces in the tables indicate that determinations for one reason or another could not be recorded.

The value of stating all the experimental findings might perhaps be open to some question. But in view of the importance of the conclusions concerning *L. acidophilus* and the paucity of data published in detail, it is hoped that such a procedure

TABLE 45

Case 43

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
2/18/23	Nothing	..	..	..	..	..	..
2/28	1000 cc. *L. acidophilus* milk	..	..	M	H	B	2
3/8	“	18	95	L	S	B	3
3/16	“	26	90	L	S	B	3
3/23	“	20	60	M	S	B	3
3/30	“	37	100	M	S	B	5
4/6	“	32	0	M	S	B	5
4/13	“	..	..	L	S	B	3
4/20	“	..	..	M	S	B	3
4/27	“	..	..	M	S	B	3
5/4	“	..	..	M	S	B	4
5/11	“	25	0	L	S	B	3
5/18	“	..	..	M	S	B	3
5/22	1000 cc. *L. acidophilus* milk + 300 grams lactose	37	0	M	S	B	2
5/31	“	40	20	L	S	Y	4
6/13	“	85	0	M	S	B	9
6/21	“	..	..	M	S	B	4
6/28	“	32	0	L	S	B	4
7/6	“	29	0	L	S	B	3
7/20	“	..	..	L	S	B	8
8/2	“	38	0	S	S	B	8
8/13	“	..	..	L	S	B	4
8/16	“	40	0	S	H	B	1
8/24	“	9	0	L	S	B	7
8/30	“	18	0	M	S	B	3
9/6	“	28	0	M	S	B	2
9/13	“	23	0	M	S	B	3
9/16	1500 cc. *L. acidophilus* milk + 300 grams lactose	..	..	..	..	..	0
9/20	“	..	..	..	..	..	0
9/27	1000 cc. *L. acidophilus* milk + 300 grams lactose	38	0	M	S	B	1
10/4	“	21	0	M	S	B	3
10/11	“	..	..	L	H	B	3
10/18	“	31	0	L	H	B	5
10/25	“	12	0	M	H	B	5
11/9	“	25	0	L	H	B	8

TABLE 45—*Continued*

Case 43—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
11/16	1000 cc. *L. acidophilus* milk + 300 grams lactose	24	95	M	H	B	2
11/26	Nothing	40	60	M	H	B	3
2/18/25	“	..	..	Incomplete			
2/19	“	..	..	..	..	..	0
3/8	“	..	..	S	H	B	5
3/12	“	..	..	Incomplete			
3/19	“	..	..	..	..	..	0

TABLE 46

Case 44

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
2/27/23	Nothing	..	..	..	..	..	..
3/26	1000 cc. *L. acidophilus* filtrate	..	..	S	H	B	1
3/30	“	42	0	..	..	..	0
4/13	“	33	0	S	H	B	1
4/17	1000 cc. *L. acidophilus* milk	..	..	..	..	..	0
4/21	“	..	..	S	H	B	1
5/18	“	14	0	M	H	B	12
6/13	“	16	40	M	H	B	15
6/28	Nothing	..	..	M	S	B	11
7/6	“	33	0	M	S	B	6
7/20	“	27	0	M	S	B	12
8/2	“	35	0	M	S	B	13
9/4	“	23	0	M	S	B	29

will more clearly define the problem and its solution. It is scarcely necessary, however, to discuss all the data in each case. Suffice it to say that certain general features are of interest. In the first place, one is immediately impressed by the comparatively low percentages of Gram-positive rods. In fact, we have in the

TABLE 47

Case 45

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
2/27/23	Nothing	..	..	..	..	..	..
3/27	500 cc. *L. acidophilus* paste	..	..	..	..	..	0
4/3	Nothing	..	..	..	..	..	0
4/10	“	..	..	..	..	..	0
4/18	1000 cc. *L. acidophilus* milk	..	..	..	..	..	0
4/24	“	..	..	..	..	..	0
5/4	“	26	0	..	..	..	0
5/25	“	11	0	..	..	..	0
5/31	“	35	0	..	..	..	0
6/28	1500 cc. *L. acidophilus* milk + 400 grams lactose	..	..	..	..	..	0
7/6	1500 cc. *L. acidophilus* milk + rectal *L. acidophilus*	85	80	..	..	..	0
9/6	“	30	0	..	..	..	0
9/13	“	20	0	..	..	..	0
10/4	“	85	0	..	..	..	0
11/4	“	..	..	..	..	..	0

TABLE 48

Case 46

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
3/10/23	Nothing	..	..	..	..	..	..
3/26	900 cc. *L. acidophilus* filtrate	22	0	..	..	..	0
4/6	“	33	0	..	..	..	0
4/13	“	16	0	..	..	..	0
4/17	1000 cc. *L. acidophilus* milk	..	..	..	..	..	0
5/4	“	34	0	S	H	B	5
5/11	“	16	70	S	H	B	3
5/25	“	33	0	S	H	B	1
6/29	1500 cc. *L. acidophilus* milk + 300 grams lactose	28	0	..	..	..	3

TABLE 48—*Continued*

Case 46—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
7/20	1500 cc. *L. acidophilus* milk + rectal *L. acidophilus*	31	5	S	H	B	1
8/16	“	85	0	S	H	B	1
8/30	“	35	0	..	..	..	0
9/6	“	25	0	S	H	B	1
9/20	“	12	20	..	..	..	0
9/27	“	31	0	..	..	..	0
10/25	“	37	0	S	H	B	3
11/16	“	33	95	S	H	B	0
11/30	“	..	..	..	..	..	0
12/3	“	..	..	..	..	..	0

TABLE 49

Case 47

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
4/22/23	Nothing	..	..	..	..	..	..
4/28	600 cc. *L. acidophilus* paste	23	0	S	H	B	0
5/22	1000 cc. *L. acidophilus* milk	..	..	M	H	B	7
5/25	“	24	99	M	H	B	3
5/29	1000 cc. *L. acidophilus* milk + 300 grams lactose	27	20	M	H	B	4
6/13	“	23	0	M	H	B	10
6/21	“	30	0	M	H	B	7
7/6	“	25	25	M	H	B	9
7/20	“	27	0	S	H	B	11
8/2	“	26	0	S	H	B	10
8/16	“	35	50	S	H	B	9
8/24	“	33	0	S	H	B	6
8/30	“	37	90	S	H	B	4
9/4	“	20	90	M	H	B	5
9/26	“	9	10	Incomplete			
9/27	“	27	0	M	H	B	1

TABLE 49—*Continued*

Case 47—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
10/4	1000 cc. *L. acidophilus* milk + 300 grams lactose	25	0	M	S	B	6
11/1	“	29	0	M	S	B	27
11/17	“	17	0	M	S	B	15
12/2	Nothing	..	..	M	S	B	5
12/4	“	18	0	Incomplete			
12/10	“	..	..	“			
2/1/24	“	..	..	M	H	B	29

TABLE 50

Case 48

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
4/17/23	Nothing	..	..	..	..	..	..
4/28	900 cc. *L. acidophilus* filtrate	..	..	..	..	..	0
5/11	“	17	0	..	..	..	0
5/18	“	..	..	..	..	..	1
5/22	1000 cc. *L. acidophilus* milk	..	..	..	..	..	0
5/29	“	..	..	S	H	B	1
6/15	“	..	..	S	H	B	4
6/29	1500 cc. *L. acidophilus* milk + 300 grams lactose	..	..	S	H	B	2
6/30	1500 cc. *L. acidophilus* milk + rectal *L. acidophilus*	..	..	S	H	B	0
7/6	“	85	10	..	..	..	0
7/20	“	85	10	S	H	B	2
8/2	“	65	0	S	H	B	5
8/16	“	85	10	S	H	B	5
8/24	“	85	80	M	H	B	3
8/30	1500 cc. *L. acidophilus* milk + 300 grams lactose	..	..	M	H	B	1
9/13	“	85	0	M	S	B	4

TABLE 51

Case 49

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
4/22/23	Nothing	..	..	..	..	..	..
4/29	1000 cc. *L. acidophilus* filtrate	..	..	..	..	..	2
5/4	"	30	0	S	H	B	2
5/11	"	30	0	S	H	B	6
5/18	"	25	0	S	H	B	4
5/22	1000 cc. *L. acidophilus* milk	31	95	S	H	B	3
5/31	"	34	65	S	H	B	7
6/13	"	54	0	M	H	B	11
6/21	"	33	0	M	H	B	5
6/28	1500 cc. *L. acidophilus* milk + 300 grams lactose	23	0	M	H	B	4
7/3	1500 cc. *L. acidophilus* milk + rectal *L. acidophilus*	85	20	S	H	B	3
7/20	"	85	10	M	H	B	11
8/4	1000 cc. *L. acidophilus* milk + 100 grams lactose	63	0	M	H	B	12
8/16	"	85	20	M	H	B	11
8/24	"	85	20	M	H	B	7
9/1	"	85	90	M	H	B	8

TABLE 52

Case 50

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
1/10/23	Nothing	..	..	L	H	B	..
1/30	1000 cc. pasteurized *L. acidophilus* milk	16	0	L	H	B	7
2/13	"	17	0	M	H	B	3
3/2	"	34	0	M	H	B	7
3/8	"	30	0	L	H	B	1
3/16	"	35	0	L	H	B	3
3/23	"	38	0	M	H	B	1
3/30	1000 cc. *L. acidophilus* filtrate	20	0	L	H	B	1

TABLE 52—*Continued*

Case 50—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
4/6	1000 cc. *L. acidophilus* filtrate	27	0	L	H	B	3
4/13	“	34	0	L	H	B	2
4/17	1000 cc. *L. acidophilus* milk	..	..	L	H	B	2
4/20	“	29	100	L	H	B	3
4/27	“	39	50	L	H	B	2
5/4	“	56	30	L	H	B	2
5/11	“	41	10	L	H	B	3
5/18	“	21	95	L	H	B	3
5/27	1000 cc. *L. acidophilus* milk + 300 grams lactose	47	0	L	H	B	3
5/31	“	40	2	L	H	B	2
6/13	“	29	0	L	H	B	4
6/21	“	41	0	L	H	B	3
6/28	1500 cc. *L. acidophilus* milk + rectal *L. acidophilus*	30	50	L	H	B	2
7/4	1500 cc. *L. acidophilus* milk + 400 grams lactose	..	..	L	H	B	2
8/2	“	36	0	L	H	B	10
8/24	“	..	20	L	H	B	7
8/30	“	85	99	L	H	B	3
9/6	“	63	0	L	H	B	2
9/13	“	34	30	L	S	B	4
9/20	“	30	0	M	H	B	3
9/27	“	..	40	L	H	B	4
10/4	“	36	0	L	H	B	3
10/11	“	23	99	L	H	B	3
10/25	“	41	50	L	H	B	6
11/9	“	62	0	L	H	B	5
11/12	“	47	0	L	H	B	1
12/4	Nothing	..	..	L	H	B	5
1/13/24	“	..	..	L	H	B	19

main been unable to confirm the high percentages claimed by Rettger and Cheplin (144). This might appear paradoxical where treatment is supposed to involve a transformation of the intestinal flora. However, it is obvious that relative percentages arrived at through a microscopic examination of fecal smears

TABLE 53

Case 51

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
1/10/23	Nothing	..	..	..	..	..	14
1/30	1000 cc. pasteurized *L. acidophilus* milk	11	0	S	H	B	3
2/13	"	14	2	S	H	B	12
3/2	"	31	0	M	H	B	16
3/8	"	33	0	M	H	B	6
3/16	"	19	0	M	S	B	5
3/23	"	22	0	M	H	B	6
3/30	1000 cc. *L. acidophilus* filtrate	34	0	M	H	B	5
4/6	"	41	0	M	H	B	5
4/13	"	39	0	M	H	B	6
4/17	1000 cc. *L. acidophilus* milk	23	98	M	H	B	2
4/27	"	48	10	S	H	B	9
5/4	"	37	25	S	S	B	5
5/11	"	50	95	S	H	B	3
5/18	"	40	0	M	H	B	5
5/27	1000 cc. *L. acidophilus* milk + 300 grams lactose	52	0	M	H	B	6
5/31	"	85	50	M	H	B	4
6/13	"	47	20	M	S	B	11
6/21	"	40	70	S	S	B	6
6/28	Nothing	38	95	S	S	LB	6
7/6	"	26	0	S	S	LB	8
8/2	"	38	0	S	S	B	21
8/13	"	45	0	M	S	B	9
8/16	"	16	0	S	H	B	3
8/24	"	40	0	S	H	B	2
8/30	"	11	0	S	H	B	5
9/6	"	38	0	S	H	B	3
9/13	"	39	0	S	H	B	4
9/20	"	55	0	S	H	B	4
9/27	"	27	0	S	H	B	4
10/4	"	30	0	S	H	B	2
10/11	"	28	0	S	H	B	2
10/18	"	27	0	S	H	B	5
10/25	"	43	0	S	H	B	3
11/9	"	40	1	S	H	B	8
11/16	"	36	0	S	H	B	3
11/21	"	42	0	S	H	B	2
12/16	"	..	..	S	H	B	13

TABLE 54

Case 52

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
1/15/23	Nothing	..	..	..	..	..	..
1/30	1000 cc. pasteurized *L. acidophilus* milk	35	0	L	H	B	5
2/13	“	37	0	M	S	B	9
3/2	“	70	0	S	S	LB	12
3/8	“	26	0	S	S	LB	4
3/16	“	40	0	M	H	B	7
3/26	1000 cc. *L. acidophilus* milk	38	0	M	H	B	7
3/30	“	23	99	M	S	B	4
4/6	“	47	100	M	S	B	7
4/13	“	38	10	L	H	B	4
4/20	“	26	95	M	H	B	6
4/24	“	..	..	L	S	B	4
4/25	Nothing	38	80	L	S	B	1
5/1	1000 cc. *L. acidophilus* milk	30	90	Incomplete			
5/18	“	34	2	M	H	B	6
5/27	1000 cc. *L. acidophilus* milk + 300 grams lactose	26	99	M	H	B	9
5/31	1000 cc. *L. acidophilus* milk	52	80	L	S	LB	3
6/13	“	15	90	L	S	LB	9
6/21	“	55	50	L	S	Y	8
6/28	Nothing	56	5	M	S	LB	6
7/6	“	27	0	L	S	B	6
8/2	“	13	0	M	S	B	12
8/13	“	23	0	M	S	B	9
8/16	“	30	5	L	S	B	3
9/6	“	45	20	M	H	B	11
9/27	“	22	0	M	H	B	9
10/4	“	24	0	S	H	B	3
10/11	“	25	10	M	H	B	4
10/18	“	12	0	M	S	B	3
10/25	“	37	50	L	H	B	3
11/2	“	35	0	M	S	B	5

cannot give a very accurate picture of the intestinal flora. In employing this method, living bacteria are indistinguishable from dead bacteria; yet it is only the viable bacteria that are of

TABLE 55

Case 53

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
1/10/23	Nothing	..	..	..	..	..	..
1/30	1000 cc. pasteurized *L. acidophilus* milk	..	..	L	H	B	7
2/5	"	35	0	L	H	B	2
2/13	"	10	0	L	H	B	4
3/2	"	45	0	M	H	B	8
3/8	"	19	0	M	S	B	3
3/16	"	32	0	M	S	B	5
3/23	"	21	0	M	S	B	4
3/30	600 cc. *L. acidophilus* paste	18	50	L	S	B	3
4/6	"	32	0	L	S	B	3
4/13	"	18	0	M	S	B	4
4/17	1000 cc. *L. acidophilus* milk	..	..	L	S	B	2
4/20	"	13	40	L	S	B	1
4/27	"	27	40	L	S	B	5
5/4	"	23	25	M	S	B	2
5/11	"	48	90	L	S	B	4
5/18	"	15	0	L	S	B	4
5/27	1000 cc. *L. acidophilus* milk + 300 grams lactose	27	99	L	S	B	8
5/31	"	85	95	L	S	LB	3
6/13	"	85	5	M	S	LB	11
6/21	"	52	50	M	S	Y	8
6/28	Nothing	..	10	M	S	LB	6
7/6	"	21	0	M	S	B	6
7/20	"	47	0	M	S	B	13
8/2	"	19	0	M	S	B	11
8/13	"	24	0	M	S	B	11
8/16	"	25	0	M	S	B	3
8/24	"	17	0	M	S	B	8
8/30	"	15	0	M	S	B	5
9/6	"	22	0	M	S	B	7
9/13	"	37	0	M	S	LB	7
9/20	"	..	0	M	S	B	7
9/27	"	17	0	M	S	B	7
10/4	"	24	0	M	S	B	6
10/11	"	18	0	M	S	B	7

TABLE 55—*Continued*

Case 53—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
10/18	Nothing	20	0	M	S	B	5
10/25	“	21	25	M	S	B	7
11/2	“	43	0	M	S	B	8
11/10	“	28	0	M	S	B	8

TABLE 56

Case 54

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
1/13/23	Nothing	..	..	..	..	..	..
1/30	1000 cc. pasteurized *L. acidophilus* milk	37	0	L	H	B	3
2/13	“	16	0	M	H	B	10
3/2	“	47	0	M	H	B	14
3/16	“	36	0	L	H	B	14
3/23	“	27	0	L	H	B	5
3/30	600 cc. *L. acidophilus* paste	20	90	L	H	B	4
4/6	“	21	5	L	H	B	6
4/13	“	23	0	L	H	B	4
4/17	1000 cc. *L. acidophilus* milk	..	..	L	H	B	3
4/20	“	19	99	L	H	B	2
4/27	“	28	0	L	H	B	5
5/4	“	28	20	L	H	B	5
5/11	“	31	60	M	S	B	3
5/18	“	27	100	L	H	B	3
5/27	1000 cc. *L. acidophilus* milk + 300 grams lactose	19	50	L	H	LB	5
5/31	1000 cc. *L. acidophilus* milk	40	60	L	H	B	12
6/13	“	32	0	L	H	B	6
6/21	“	40	0	L	H	B	4
6/29	1500 cc. *L. acidophilus* milk + 400 grams lactose	..	..	L	H	B	4

TABLE 56—*Continued*

Case 54—Continued

DATE	TREATMENT	MICROSCOPIC L. ACIDOPHILUS	PLATE L. ACIDOPHILUS	FECES			NUMBER OF NORMAL DEFECATIONS
				Amount	Consistency	Color	
		per cent	*per cent*				
7/4	1500 cc. *L. acidophilus* milk + rectal *L. acidophilus*	..	..	L	H	B	5
8/2	"	27	0	M	S	LB	9
8/13	"	50	..	M	S	LB	2
8/30	1500 cc. *L. acidophilus* milk + 400 grams lactose	59	75	M	S	LB	4
9/6	"	45	80	M	S	B	3
9/13	"	35	90	S	S	B	1
9/20	"	37	95	M	S	B	2
9/27	"	36	0	M	S	B	3
10/4	"	48	0	M	H	B	3
10/11	"	31	0	M	S	LB	5
10/18	"	25	80	M	S	LB	3
10/25	"	41	70	M	S	LB	4
11/2	"	39	80	M	S	B	7
11/9	"	53	5	M	S	B	6
11/6	"	30	90	M	S	B	2
11/23	"	29	40	M	S	B	3
12/4	Nothing	..	..	M	S	LB	5
1/1/24	"	..	..	M	S	LB	15
2/1	"	..	..	M	S	LB	20
3/1	"	..	..	S	H	B	15
4/1	"	..	..	S	H	B	13
5/1	"	..	..	M	H	B	15
6/1	"	..	..	S	H	B	18
7/1	"	..	..	S	H	B	14
8/1	"	..	..	S	H	B	11

significance. This is amply confirmed by a second generalization that may be deduced from these tables; namely, that not even the slightest agreement exists between the microscopic and plate counts. The plate count itself is a none too accurate index of the relative percentages of Gram-positive and Gram-negative organisms in the intestinal tract. To begin with, the medium is selective; being definitely employed for the purpose of encouraging the growth of *acidophilus*-like colonies. This means the suppression of large numbers of other organisms not so favored. It is not unreasonable to say, then, that the microscopic count of the relative percent of viable *L. acidophilus* is likely to be an underestimate, while the plate count is likely to be an overestimate, of the true balance. Finally, relative percentages are unsatisfactory at best for they are not an adequate criterion of what actually occurs in the intestine. A high relative per cent of Gram-positive organisms might be taken to mean a transformation of the intestinal flora, in that Gram-negative organisms had been suppressed or eliminated, whereas strictly speaking there might be just as many such organisms as existed prior to the influx of relatively large numbers of Gram-positive organisms. We attempted to carry out an investigation of the *actual* numbers of Gram-positive and Gram-negative organisms in the feces of patients under treatment but the results were too variable to permit of generalizations, consequently these data remain unpublished. To obtain adequate results in such a study it would be imperative to ascertain the per cent of moisture in all fecal specimens examined bacteriologically so that proper calculation might be made. This was not possible at the time, but is now being carried out.

Despite the limitations mentioned the plate counts are a more satisfactory index of the transformation of the intestinal flora than the microscopic counts. A high *L. acidophilus* plate count is almost invariably accompanied by an increase in the number of normal defecations; although it is not possible to predict that an increase in number of normal defecations will always be accompanied by an increase in the relative per cent of *L. acidophilus* by plate count. From a clinical standpoint this is of utmost significance, for it means that a transformation of the intestinal flora from a Gram-negative to a Gram-positive type is a guarantee of the alleviation of constipation. It implies also that the mere administration of *L. acidophilus* cannot in itself be regarded as a sure cure, although it will prove efficient in most cases of constipation.

The data on the character of the feces are of real practical value. A change in the character of the feces is usually accompanied by an increase in the number of normal defecations; although it must be stated that the alleviation of constipation is

TABLE 57

Transformation of intestinal flora as influencing constipation

CASE NUMBER	BEFORE TREATMENT		NUMBER OF NORMAL DEFECATIONS IN 30 DAYS FOLLOWING TRANSFORMATION OF FLORA
	Number of normal defecations	Number of days	
3	3	14	24
5	7	14	28
18	49	92	23
30	9	34	18
35	1	14	28
36	1	11	22
41	3	7	29
42	3	15	27
47	0	6	25
50	6	19	11
51	14	21	20
52	5	16	25
53	7	21	27
54	10	17	18

TABLE 58

The recovery of L. acidophilus from feces after discontinuing treatment

CASE NUMBER	L. ACIDOPHILUS	NUMBER OF DAYS AFTER TREATMENT
	per cent	
16	64	154
16	10	374
19	70	92
19	5	120
29	5	61
34	75	56
34	25	69
34	5	542
35	25	550
40	10	62
40	2	83
41	90	105
41	40	127
42	50	110
51	1	133
52	50	119
53	25	119
64	50	111

not always accompanied by a change in the character of the feces. Under *L. acidophilus* treatment the feces tend to become larger in amount, softer in consistency and lighter in color. In fact there is some basis for believing that a correlation exists between the transformation of the intestinal flora and the change in character of the feces. In general, when the character of the feces becomes markedly favorable the relative per cent of *L. acidophilus* by plate count is higher. Therefore, it is of importance to observe closely the character of the feces during the administration of *L. acidophilus.*

To indicate more clearly that the transformation of the intestinal flora is accompanied by a marked increase in the number of normal defecations, table 57 has been arranged. The term "transformation" has here been limited to a relative percentage of 98 to 100 per cent *L. acidophilus* by plate count. It might have been admissible to regard percentages lower than 98 as still indicating "transformation" but we prefer the more rigid criterion. It will be seen that with only three exceptions there has been an increase in the numbers of normal defecations where the intestinal flora has been transformed as compared with the period before treatment.

The permanence of the therapeutic results obtained by *L. acidophilus* treatment have already been discussed. But the persistence of viable *L. acidophilus* organisms in the human intestinal tract is also of importance. In order to throw further light on this point a series of reëxaminations was made of fecal specimens after treatment was discontinued for about two months or more. The results of those plate counts are recorded in table 58.

It will be seen from table 58 that it is possible to recover *L. acidophilus* in appreciable amount long after treatment has been discontinued. In fact, in a majority of instances cited, *L. acidophilus* was recovered in percentages as high as 90, more than three months after discontinuing treatment. Of these, two cases harbored *L. acidophilus* for over a year and a half after treatment, and one over one year, in rather small amount. In nineteen cases no *L. acidophilus* were recovered from the feces on reëxamination.

In this connection may be mentioned some unpublished results very generously placed at our disposal by Dr. H. Tissier of the Pasteur Institute, Paris, concerning two healthy individuals over 45 years of age on a vegetarian diet for 18 years after investigation of *L. acidophilus* and *B. bifidus:*

		per cent
I.	*B. coli*	2.2
	Enterococcus	13.2
	B. mesentericus	2.2
	L. acidophilus	79.2
	B. exilis	3.2
II.	Streptococcus intestinalis I and II	10
	L. acidophilus	5
	B. exilis	15
	B. bifidus	70

It is apparent from these and our own data that *L. acidophilus* and allied organisms may persist in the human intestine long after specific ingestion has been discontinued. The results in table 58 confirm the clinical observations mentioned in part III of Chapter V concerning the permanence of the results obtained by *L. acidophilus* therapy. It will be noted that seven of the twelve patients cited in table 58, namely, cases 16, 29, 40, 41, 42, 52 and 53 were previously referred to as cases in whom the beneficial effects persisted after *L. acidophilus* treatment was discontinued. There is evidence, therefore, for concluding that it is not necessary to continue indefinitely the administration of *L. acidophilus* in order to maintain the beneficial effects secured. This will be discussed more fully from a clinical standpoint in chapter IX.

To summarize briefly the results presented in this chapter:

1. Despite the limitations of the microscopic and plate counts of *L. acidophilus* there is evidence to indicate that the ingestion of this organism in large numbers tends to transform the intestinal flora from a Gram-negative to a Gram-positive type.
2. A high relative per cent of *L. acidophilus* by plate count is almost invariably accompanied by an increase in the number of normal defecations, although the converse is not always true.
3. A change in the character of the feces to a larger amount, softer consistency and lighter color is almost invariably accompanied by a high relative per cent of *L. acidophilus* by plate count.
4. It is possible to recover *L. acidophilus* from the feces of patients long after treatment has been discontinued.

CHAPTER VII

THE NATURE OF L. ACIDOPHILUS THERAPY[1]

The relief from chronic constipation and diarrhea by the ingestion of milk fermented with *L. acidophilus* is a phenomenon worthy of further study. The beneficial effects obtained by such therapy might be due to a multiplicity of factors. It has been held that chief among these is the ingestion of an increased volume, and more particularly the addition of considerable water to the daily diet. As against this physical or mechanical interpretation, there is a chemical explanation, based on the presence of a considerable amount of lactic acid, together with the disintegration products of the organism *L. acidophilus*. Finally, there is the strictly bacteriologic interpretation, which holds the living *L. acidophilus* organisms responsible for all benefits. The experiments here described, which are still in progress, were devised in the hope of arriving at some definite conclusion regarding this phenomenon.

EXPERIMENTAL DATA

Patients with a history of chronic constipation were given the carmin test, and daily defecations were recorded for some time prior to any treatment, in order to establish the patient's intestinal motility. The feces were examined microscopically, and the presence of Gram-positive and Gram-negative organisms was determined by a modified method described in Part III of the Appendix. Some of the patients were then given, during the period of one week, daily doses of 1 liter of skim milk sterilized at 20 pounds pressure for fifteen minutes. This uninoculated milk is identical with that ordinarily inoculated with *L. acidophilus;* therefore if increased volume is of prime importance, beneficial effects should be induced. The data are summarized in table 59.

The heading "no treatment" refers to the period of time during which the patients have been under observation prior to any treatment. In the first two columns under this heading are given the number of days during which the patient had normal defecations. The second column gives the total number of days under observation. The next heading "sterile milk," is similarly subdivided to show the number of days during which normal defecations occurred while the patients were ingesting milk sterilized in the manner just described.

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[1] Reprinted in part from Jour. Am. Med. Ass., *80*, March 3, 1923, 602–604, and Arch. Int. Med , *33*, January, 1924, 55–57.

It is readily apparent that most of the these patients were severely constipated; eight of the ten patients had one normal defecation per week, or less. When these patients were given daily 1 liter of sterile milk, it will be seen that there was little, if any, improvement in their condition. While case 27 did show some improvement, case 33 became much more constipated. Case 29 continued to be severely constipated. Case 30 showed a slight improvement, while case 34 became somewhat more constipated.

TABLE 59

Occurrence of defecations in constipated patients under different treatments

CASE NUMBER	NO TREATMENT		STERILE MILK		PASTEURIZED L. ACIDOPHILUS MILK		REGULAR L. ACIDOPHILUS MILK			AFTER L. ACIDOPHILUS TREATMENT			
										Lactose		Nothing	
	Number of normal defecations	Number of days	Number of normal defecations	Number of days	Number of normal defecations	Number of days	Number of defecations first 7 days	Number of normal defecations	Number of days	Number of normal defecations	Number of days	Number of normal defecations	Number of days
27	2	14	3	7	2	5	5	46	62	..	..	25	80
33	5	19	2	7	1	6	6	17	22	..	..	259	350
29	0	36	0	7	..	..	4	106	149	9	13	103	170
30	9	34	3	7	..	..	4	46	70	52	65	64	76
34	12	20	4	7	2	5	6	30	62	..	..	43	105
31	1	7	..	..	2	6	6	22	63	99	154	33	71
39	3	21	..	..	0	12	7	13	15	54	62	16	21
35	1	14	..	..	0	7	3	47	104	..	..	..	..
36	1	11	..	..	0	7	2	30	52	..	..	..	..
32	0	14	..	..	0	7	2	151	241	..	..	..	..

For the following week, three of these patients, as well as five others, were given daily 1 liter of skim milk, sterilized as described, but inoculated and incubated for twenty-four hours with *L. acidophilus* in the usual manner, after which it was pasteurized by being heated in the Arnold sterilizer (steam at atmospheric pressure) for ten minutes. In order to avoid extreme coagulation of the casein, which would make the milk indigestible, the acidity caused by the fermentation was first neutralized with normal sodium hydroxid. The milk was plated out on whey agar after pasteurization, and was found to be sterile.

From the data that are presented in table 59, it will be seen that there was no improvement in the intestinal condition of any of these patients when they were taking pasteurized milk, over their condition when taking sterile milk. Also, it may be seen that pasteurized *L. acidophilus* milk had a constipating effect, if any. It is realized that the neutralization of the milk prior to pasteurization prevents any possible activity of the lactic acid which was formed during fermentation. Therefore, in continuing these experiments, this deficiency has been overcome by adding lactic acid in an equivalent amount to the milk after pasteurization, with the same results. It is impossible completely to separate the chemical from the bacteriologic factors in such therapy, for the phenomenon must be biochemical, in the sense that the bacteria secrete by-products of a chemical nature. Nevertheless, it is of interest to determine, if possible, whether these by-products are of most importance or whether the most important factor is the actual viable *L. acidophilus* in the intestine. With this end in view, patients have been given the Berkefeld filtrate of *L. acidophilus* milk, which contains the essential acids, salts and enzymes, but no living bacteria. These results will be discussed presently.

Since neither the physical nor the chemical interpretation of *L. acidophilus* therapy can be considered adequate, the patients were given daily 1 liter of the regular *L. acidophilus* milk. It contained approximately 200,000,000 viable organisms in each cubic centimeter. It will readily be seen from table 59 that the beneficial effects of this treatment were soon in evidence, and that relief from chronic constipation was obtained. This indicates that the phenomenon is strictly bacteriologic in nature. Under the heading "regular *L. acidophilus* milk," there are the usual subdivisions, but the first column contains the number of normal daily defecations occurring in the first seven days. This was inserted as a basis of comparison with the results obtained with sterile and pasteurized milk. There has been an improvement, often striking, in every patient studied. This is further substantiated by the persistent improvement shown under continued treatment, as evidenced by the data in the remaining columns under this heading. Frequently the patient had more than one movement a day; but, in order to leave no room for question, they have not been counted here. Thus in all the columns marked "number of normal defecations," it is to be understood that the number of days during which one or more movements occurred is recorded rather than the actual number of defecations.

TABLE 60

Percentage of defecations under different treatments

CASE NUMBER	NO TREATMENT	STERILE MILK	PASTEURIZED L. ACIDOPHILUS MILK	REGULAR L. ACIDOPHILUS MILK		AFTER L. ACIDOPHILUS TREATMENT	
				First 7 days	Total	Lactose	Nothing
27	14	43	40	71	74	..	61
33	26	30	17	86	77	..	73
29	0	0	..	57	71	70	60
30	26	43	..	57	66	80	84
34	60	57	40	86	49	..	41
31	15	..	33	87	35	64	32
39	15	..	0	100	87	87	76
35	8	..	0	43	45	..	..
36	9	..	0	30	58	..	..
32	0	..	0	30	63	97	..

An interesting comparison is made in table 60 on a percentage basis, between the period before and after *L. acidophilus* therapy the number of days of observation being used as a divisor, and the number of normal daily defecations as a dividend. If the figures under the heading "regular *L. acidophilus* milk" are compared with those in the preceding columns, it will be noted that a marked increase has occurred. For example, in the first two cases, 27 and 33, the percentage of defecations is about double that obtained with sterile or pasteurized *L. acidophilus* milk. In the last four cases, 39, 35, 36, and 32, this increase is still greater. Quantitative data such as these may, in certain instances, yield a distorted emphasis, since the character of the stools is frequently as important as the number of defecations. Therefore, it is all the more significant that the character of the stools from patients under intensive *L. acidophilus* therapy is almost invariably more nearly normal than when there is no treatment, or sterile milk or pasteurized *L. acidophilus* milk is given. The stools are of a softer consistency, and the color is much lighter. On the other hand, certain limitations are recognized, chief among these being that the size of each stool is frequently as important as the number of defecations. While *L. acidophilus* therapy has a general tendency to increase the size of the individual stools, frequently the movements increase only in number, and in some patients the size remains unsatisfactory. These considerations, are difficult to tabulate, but should receive due recognition in the general evaluation of this method of treatment. In table 8 data showing the permanence of benefits induced by *L. acidophilus* treatment have already been presented.

Of further interest is another experiment that is corroborative of the fact that the action of *L. acidophilus* is a strictly bacteriologic phenomenon.

Two members of the Psychiatric Institute staff were extremely susceptible to *L. acidophilus* milk. Diarrhea followed the ingestion of comparatively small amounts. Therefore, it was determined to give them each 400 cc. of *L. acidophilus* milk which had been pasteurized, without their knowledge in order to eliminate so far as possible any psychologic factors. It is particularly significant that the reactions of these two persons were identical. Following the ingestion of the milk, there was a certain amount of gas formed. This was not accompanied by undue tension or pain. Instead of having three or four defecations, as had previously been the case when 400 cc. of the regular *L. acidophilus* milk was taken, each subject had only one normal defecation, which was customary, before retiring. Three days after this test, each subject was again given 400 cc. of regular *L. acidophilus* milk, unpasteurized. Both were under the impression, however, that it had been pasteurized. Neither had any defecation, and both complained less of gas formation than previously with the pasteurized milk. Again, they were given regular *L. acidophilus* milk in greater volume, 800 cc., under the impression that it was pasteurized although it was not. With this amount, both patients had a diarrheal attack, curiously enough each recording three defecations, one of these being semisolid, and one fluid. As a further check, these subjects were given 800 cc. of pasteurized *L. acidophilus* milk, they being under the impression that it was the regular *L. acidophilus* milk which gave them diarrhea. Their reactions were again identical. Each had one abundant semifluid movement at about 6 p.m., but no more. Apparently, there was some stimulation of peristalsis, but the results were not comparable to the effects produced by the regular *L. acidophilus* milk.

It has been shown that the action of *L. acidophilus* milk is neither strictly physical nor chemical, that is, does not depend upon volume or chemical constituents, but appears to be essentially a bacteriologic phenomenon. This conclusion was based chiefly on the fact that neither sterile skim milk nor sterilized *L. acidophilus* milk influenced constipation, as did *L. acidophilus* milk in which the viable organisms were uninjured. The foregoing experiments were open to some criticism on the ground that sterilizing *L. acidophilus* milk caused a change in chemical composition. The study was therefore extended along somewhat different lines.[2]

In order to feed *L. acidophilus* milk in which the viable organisms were eliminated, the following procedure was adopted: *L. acidophilus* milk prepared in the usual way was centrifuged, and the supernatant liquid was put through a Mandler diatomaceous filter. The labor involved was time-consuming. It was through the courtesy of Dr. Charles Krumwiede, assistant director of the Research Laboratories of New York City Health Department that we were able to use a Sharples supercentrifuge, which greatly facilitated the preliminary work. The liquid was run through a "Vaccu-Filter," then through a coarse Mandler diatomaceous filter, and finally through a fine Mandler filter. This filtrate was always tested for sterility on whey agar plates and was fed to patients as *L. acidophilus* "filtrate," 900 cc. per day. This amount is roughly equivalent to the liquid portion of 1000 cc. of regular *L. acidophilus* milk. An attempt was made to feed the residue from the supercentrifuge which contained viable *L. acidophilus* organisms, for comparison with the filtrate;

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[2] For this suggestion I am indebted to Dr. H. C. Ward, Director of the Public Health Department, Toledo, Ohio.

but the supply was not continuous, and therefore the results though negative, were inadequate. Previously in stating the results of feeding sterilized *L. acidophilus* milk, mention was made of the fact that the acidity of *L. acidophilus* milk was neutralized prior to sterilization. This procedure obviously affected the lactic acid content. Consequently, in continuing the feeding of sterilized milk in the present study, chemically pure lactic acid was added to sterilized *L. acidophilus* after sterilization in an amount equivalent to the acidity as previously titrated by twentieth normal sodium hydroxid. In accordance with the general plan of our *L. acidophilus* studies, the subjects were psychotic patients, cases 50 to 54 being male and the others female. Observations as to daily defecations were recorded prior to treatment.

TABLE 61

The influence of constipation of L. acidophilus in various forms

CASE NUMBER	BEFORE TREATMENT		STERILIZED L. ACIDOPHILUS MILK + LACTIC ACID		L. ACIDOPHILUS FILTRATE		L. ACIDOPHILUS MILK	
	Number of normal daily defecations	Number of days	Number of normal daily defecations	Number of days	Number of normal daily defecations	Number of days	Number of normal daily defecations	Number of days
44	1	27	..	..	1	21	9	21
46	0	16	..	..	2	21	6	21
65	11	31	..	..	5	14	..	..
48	0	11	..	..	0	13	..	..
29	0	36	0	7	6	36	13	24
37	19	45	6	21	16	38	7	23
49	2	7	..	..	12	19	72	92
50	7	21	8	21	8	22	10	21
51	14	21	17	21	16	22	19	22
52	5	16	14	21	..	..	17	23
53	7	21	9	21	..	..	11	21
54	10	17	16	21	..	..	12	21

In table 61 are presented the results as obtained with *L. acidophilus* milk prepared in the manner described above. Considering first the influence of "*L. acidophilus* filtrate" on constipation, it will be seen that in general there is little, if any, difference in the relative number of normal defecations in the period during which *L. acidophilus* filtrate was ingested as compared with the period before treatment. This is to say, that the chemical constituents of *L. acidophilus* milk, including the acid reaction, are not effective in alleviating constipation. Neither does the physical factor of volume exert much influence, since the patients received 900 cc. of *L. acidophilus* filtrate daily. The patients were subsequently given regular *L. acidophilus* milk, and it will be seen that with one exception all patients showed some increase in the number of normal defecations. This is not as striking as might perhaps have been anticipated, but it must be remembered that the patients had been permitted to become extremely constipated before administering *L. acidophilus* milk.

These data, therefore, lend further support to the conclusion that *L. acidophilus* therapy is more strictly bacteriologic than it is chemical in nature. It is manifest that any bacteriologic phenomenon may be regarded as biochemical in character, consequently that of *L. acidophilus* is no exception. We have tried to determine where the chief emphasis belongs. Are the laxative qualities of *L. acidophilus* milk due mainly to the physical factor of volume? We have shown that this is not the case. Are they due to the chemical constituents elaborated by bacteria? These experiments would tend to disprove that contention. Are they due chiefly to the transformation of the intestinal flora as here indicated, and therefore essentially bacteriologic in nature? Evidence of this may be found in data already presented, namely, that the beneficial effects of *L. acidophilus* persist for a considerable period of time after discontinuing treatment. Also it is important to note that viable *L. acidophilus* organisms have been recovered from the feces of patients long after discontinuing treatment.

Again, when the results of feeding sterilized *L. acidophilus* to which lactic acid has been added, are considered, it will be seen that there is little evidence of any relief of constipation during this period as compared with the period before treatment. The physical factor of an increased volume was present of course. However, when regular *L. acidophilus* milk was added to the diet, in all but one instance an increase in the number of normal defecations was noted While this increase is not striking, when all the facts are surveyed they indicate that it is a bacteriologic rather than a chemical interpretation that will account for the action of *L. acidophilus.* Again it will be remembered that when *L. bulgaricus* milk was fed in the same amount as *L. acidophilus* milk, no marked alleviation of constipation occurred. There was present the physical factor of volume; and chemically a greater degree of acidity. Thus, where the acidity of 10 cc. of an average 36 hour culture of *L. acidophilus* was neutralized by 13.2 cc. of N/20 NaOH, it required an average of 23.3 cc. of N/20 NaOH to neutralize the acidity of 10 cc. of a 36 hour culture of the three strains of *L. bulgaricus* milk employed in the feeding experiment described. The acidity of the *L. bulgaricus* milk, therefore, was practically double that of the *L. acidophilus* milk. Therapeutically the latter was incomparably superior to the former, indicating that the physical and chemical factors were not of as great importance as the bacteriologic in alleviating constipation. Again, it should be remembered that the addition of lactose to *L. acidophilus* milk during feeding does not markedly increase the benefits. Undoubtedly the presence of considerable lactose in the intestine results in a larger production of lactic acid, yet this chemical factor does not appear to be of particular significance when viewed from the standpoint of therapy. Lactose, however, is valuable in making permanent the benefits of *L. acidophilus* treatment, thus indicating that it influences the growth of *L. acidophilus* organisms, or the bacteriological factor involved.

It has been shown that *L. acidophilus* affects elimination from the human intestinal tract, but to explain the nature of this phenomenon is a far more difficult problem. Evidence has been presented that such activity seems to depend more upon the bacteriologic than the physical or chemical factors involved. The presence in the intestine of *L. acidophilus* in large numbers may conceivably make conditions inimical to the growth of putrefactive microörganisms. Definite proof of an actual reduction in the numbers of putrefactive organisms is, however, still lacking, and therefore this remains one of the most interesting of the unsolved problems.

Considering further unsolved problems which might throw light upon the nature of *L. acidophilus* therapy, the following speculations are advanced: (1) *L. acidophilus* may secrete enzymes which act directly upon the intestinal mucosa to stimulate peristalsis. (2) *L. acidophilus* may secrete or excrete substances toxic to putrefactive organisms. (3) *L. acidophilus* may change the hydrogen ion concentration of the intestinal contents to such a degree as to inhibit the growth of putrefactive organisms. (4) *L. acidophilus* may change the hydrogen ion concentration of the intestinal tract to such a degree as to stimulate peristalsis directly. (5) *L. acidophilus* may produce lactic acid of chemical structure different from inactive lactic acid or that produced by other microörganisms usually present in the intestine which may directly stimulate peristalsis.

Summarizing the experiments discussed in this chapter the following results may be noted:

1. The action of *L. acidophilus* is not a physical phenomenon, since patients receiving sterile milk were not relieved of constipation.
2. The action of *L. acidophilus* does not appear to be a strictly chemical phenomenon, since patients receiving pasteurized *L. acidophilus* milk were not relieved of constipation.
3. In order to further study the influence of physical and chemical factors, *L. acidophilus* milk was centrifuged and run through a Mandler diatomaceous filter. Thus the chemical constituents were little altered. When fed to constipated patients this filtrate was practically without effect. Regular *L. acidophilus* milk ingested subsequently resulted in an increase in the number of normal defecations.
4. *L. acidophilus* milk was sterilized and lactic acid added, thus again approximating its original chemical composition. When feed to constipated patients, little change was noted. Regular *L. acidophilus* milk ingested subsequently resulted in an increase in the number of normal defecations.
5. These data indicate that *L. acidophilus* therapy is essentially bacteriologic rather than physical or chemical in nature.

Plate IV. Microphotograph of Gram-stained L. acidophilus

Taken with Zeiss oil immersion, apochromat, and projection ocular No. 4.

CHAPTER VIII

INDICAN AS INFLUENCED BY CONSTIPATION AND L. ACIDOPHILUS THERAPY[1]

Constipation has been defined by Osler to be "the retention of feces from any cause." It is generally assumed that constipation is accompanied by intestinal putrefaction. However, the measure of putrefactive processes has always proved a baffling problem. Indican, perhaps, has been most frequently regarded as an index of putrefaction. The significance of indican in the urine has been the subject of controversy for many years, and the literature is correspondingly inconsistent. One may follow the pendulum from the point where indican in the urine is always considered abnormal to the opposite point where its presence in considerable amount is anticipated in good health. Ever since Baumann's (18, 19) early work, the tendency of those who consider indican evidence of a pathological condition has been to ascribe to it the responsibility for such diverse disorders as Rigg's disease (115) gingivitis, diseases of the eye (55), even psychotic states. On some points there is general agreement, however.

That tryptophan is a precursor of indican has been proven by the work of Underhill (177), Asayama (3), Hopkins (83), and others (118, 128). It is generally accepted that the tryptophan is derived from the ingested proteins, and is under ordinary circumstances completely oxidized. In delayed absorption, however, it is subjected to the action of the putrefactive bacteria of the intestine, forming indol which is oxidized to the soluble indoxyl, absorbed and paired with sulphuric acid, probably in the liver (79), and being excreted in the urine as potassium indoxyl sulphate or indican. The only other recognized source of indican is a pathological suppurative process, e.g., putrid abscess, retention of dead foetus, gangrene, etc. It has been shown by Baar

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[1] Reprinted from Jour. Metabolic Res., with minor changes by special courtesy of the author, Lillian Segal, and the editor, Dr. F. M. Allen.

(6) that any operation which involved cutting the intestinal wall was accompanied by excessive excretion of indican which lasted for about a week after the operation.

Much work has been done to prove that indican is the result of putrefactive decomposition in the intestine. Folin (66, 67) in his noteworthy experiments on the chemical composition of the urine, states: "The urinary indican is not to any extent a product of the general protein metabolism; it is therefore probably, as is generally supposed, a product of intestinal putrefaction, and may consequently be assumed to indicate approximately the degree of putrefaction in the intestinal tract." If it is accepted, as is claimed by most investigators that indican is formed by bacterial decomposition of proteins, then since the conceded definition of putrefaction is bacterial decomposition of proteins, it follows by definition that the indican excretion is an index of intestinal putrefaction. There has been some attempt to show that indican may be a product of normal cell metabolism (23, 180). However, none of this work was very scientifically controlled and much of it was shown faulty by Ellinger (60).

It was a simple step from the conception of indican as the product of intestinal putrefaction to the idea of indican as a cause of auto-intoxication (84, 137). And what more favorable circumstance for the excessive formation of indican than constipation with its retention of protein masses? The frequent occurrence of blue color in the urine of constipated individuals in response to qualitative indican tests, combined with so logical an explanation of auto-intoxication, laid the foundation for the various theories which ascribe to indican many malevolent and far-reaching effects. Both "constipation" and "auto-intoxication" are terms which permit of no exact definition. The general meaning of constipation is an infrequency of bowel movements; the general symptoms of auto-intoxication are foul breath, coated tongue, indifference to and often refusal of food, marked constipation, lassitude and headache. When we consider that in addition to the vagueness of the terms, the indican reaction itself was in no scientific sense quantitative, it seems difficult to understand how a theory with so little controlled and experimental support could be so frequently asserted and accepted. The indican reaction was also obtained in diarrhea, sprue, and other intestinal disorders.

The interpretations of indican findings have been many, but it has most frequently been held responsible for psychotic and neurotic states. Matson (117), supported by Stewart, Gillespie, McCall and Taylor in discussion, ascribes neurasthenia, migraine, and delusional states to indicanuria. Townsend (176), Coriat (49), Pilcz (135), Bruce (26), Cleghorn (41), Taubert (167), and others have found what they considered significant indican values in the functional psychoses; and yet there is very little agreement either as to the relation between the indican excretion and the mental state, or to the type of psychosis of which excess elimination is characteristic. Pilcz finds excess indican in both phases of manic-depressive insanity; Bruce finds an excess in depressions; Coriat's low figures occur in the melancholia of the involution period, while Townsend's highest results come in melancholia with agitation. Levine (112) in an examination of 837 urines frequently obtained "pathological" findings for psychoneuroses, but since he quotes no figures it is difficult to estimate how high or pathological these findings were.

The evidence for the opposition is not so plentiful but of a more satisfactory scientific character. The outstanding work has been done by Folin (64, 65) in a carefully planned and controlled series of metabolism experiments on psychiatric material. He concluded that it was "impossible to identify any one metabolism peculiarity with any particular form of mental disorder." Although his indican determinations were not exactly quantitative, they were carefully compared with a color standard and showed that no correlation existed between the indican excretion and the mental state. Borden (24) using a more convincing quantitative method concluded that "indol bears no constant relation causative or other to any particular form of insanity." Osler (132) regards indicanuria as a common condition, not especially connected with constipation, while Richard C. Cabot in a discussion of Daland's (51) paper claims that "indicanuria gives us no diagnostic or therapeutic indication." Morgan (122) and Hurst (88) object to associating indicanuria with common chronic constipation, the former linking it only to pathological conditions, as obstipation of the transverse colon, peritonitis, etc. In a most extensive series comprising about 15,000 examinations in all, Baar (6, 7, 8, 9) found true intestinal auto-intoxication very rare. Indeed, indican appeared oftener in the urine of non-constipated than of constipated cases. His theory is that the quantity of indican entering the blood is not so much a factor of the amount of intestinal putrefaction as of the integrity of the intestinal mucosa. Comparatively mild intestinal putrefaction will produce auto-intoxication if the mucosa, the so-called lines of defense, are broken down. This condition may exist unsuspected in mild form and may be a much frequent cause of indicanuria than has been supposed. Daremberg (52) found a close connection between indicanuria and kidney disturbance.

Parallel with the general disagreement as to the etiology of indicanuria is the attitude toward the toxicity of indol. Underhill and Kapsinow (178) injected indol and skatol subcutaneously into dogs with no effect. Baar (6) does not regard indoxyl compounds as toxic but thinks they accompany the formation of other compounds, as the ptomaines, and so should be carefully watched. The experiments of Richards and Howland (149) show that when the oxidative powers of the organism are depressed, preventing the oxidation of indol to indoxyl compounds, otherwise insignificant subcutaneous injections of indol are highly toxic. Indol administered by mouth (Herter 76, 79), however, was tolerated beyond the amount that would be absorbed from the intestine in the severest pathological condition. He found that the continued administration finally produced headache, irritability, etc. It might seem, therefore, that only when the cells most concerned in oxidation have been structurally damaged allowing indol to enter the circulation is there any danger of intoxication. This may account for the varying effects of intestinal putrefaction.

Following the idea that indican is a product of protein cleavage by putrefactive bacteria there have been numerous investigations on controlling indicanuria through the diet and the resulting effect on intestinal flora. Hirschler (81) found that he could inhibit the production of indol, phenol, and the oxyacids in a protein mixture by adding sucrose, starch, dextrin, or lactose. This worked out perfectly in the test tube, but unfortunately his results on animals were less satisfactory. Herter and Kendall (78) succeeded in influencing the intestinal flora by abrupt changes in the chemical composition of the food. They found with monkeys and kittens that a high protein diet stimulated the growth of proteolytic flora, while high carbohydrate diet caused the aciduric organisms to flourish. The aciduric domination was also accompanied by a reduction in indol, skatol, phenol, etc., in these experimental animals. Torrey (171) found that he could influence the intestinal flora to become more aciduric by feeding a high carbohydrate diet, but the degree of transformation depended largely on the type of flora present before treatment. MacNeal, Latzer, and Kerr (116) in a careful examination of normal fecal bacteria had found about 70 per cent Gram-negative organisms of many different kinds. Rettger and Horton, (146) however, found that on any standardized diet certain types predominate, and if a large per cent of the diet is carbohydrate, *L. acidophilus* and *Bacillus bifidus* will be present in large number. Turning from the bacteriological effect of diet once more to its biochemical influence, we find many reports of decreased indican excretion on high carbohydrate diets. Simpson (160) in a case of tetany of intestinal origin obtained high indican values when he fed a high protein diet, and low indican values on a high carbohydrate diet. Underhill and Simpson (179) experimenting with various diets found that the excretion of indican and phenol varied directly with the protein intake. If constipation set in, however, this effect was overshadowed, and the indican excretion was increased regardless of the diet. They concluded that in any attempt to regulate intestinal putrefaction through the diet, a careful study must be made either of the indican excretion or of the fecal flora.

The present investigation is an attempt to correlate several of these factors. It was undertaken to determine:

1. What effect the alleviation of chronic constipation had on indican excretion.
2. What effect a transformation of the intestinal flora from the proteolytic to the aciduric type had on indican excretion.
3. What effect diminished indican excretion had on psychotic conditions.

The work was done on psychotic patients who had been fed *L. acidophilus* preparations for some time as described in the foregoing chapter. There were twenty-three patients receiving the treatment, and twenty-five controls. It was not thought necessary to use normal people as controls since we were primarily interested in the effect of changing the intestinal flora on the indican excretion, and the resulting effect, if any, on the psychotic patients. The normal value for indican excretion, 5 to 20 mg. daily, had been so uniformly agreed upon in the literature that it was accepted as being representative. It was considered inadvisable to determine phenols after Tisdall's (168) critical analysis showing the non-specificity of our present methods. The subjects of this study were on the clinical service of the Psychiatric Institute. They were all patients admitted to the Manhattan State Hospital, and they received the routine hospital diet. Twenty-four hour specimens of the urine were collected and the following records were kept: volume, specific gravity, color, reaction, indican, kind and amount of food consumed by each patient preceding and during collection of urine, a record of the bowel movements, and microscopic and plate counts of fecal flora. The specific gravity and color records are omitted from the tables for lack of space, as they coincided with general expectations, i.e., generally 1.010 to 1.020 although occasionally dropping as low as 1.002 or rising as high as 1.029. The readings were always consistent with the color and volume; large volumes of urine being light colored and of low specific gravity, whereas the urines of low volume were dark colored and of high specific gravity. The twenty-four hour specimens were started before breakfast after discarding the first morning urine, kept in a cold place during the twenty-four hours and analyzed as soon as collection was completed. Toluol was used as a preservative, as formaldehyde has been found to interfere with the indican test (53). Total acidity was determined by Folin's method. The feces were examined microscopically and the presence of Gram-positive and Gram-negative organisms was determined in the bacteriological laboratory by a modified method described in part III of the Appendix. The indican method chosen was that described by Myers and Fine[2] (127) because quantitatively it compared very favorably

[2] Method: *a*. Qualitative test: About 10 cc. of faintly acid urine are shaken with a small amount (0.1 gram) basic lead acetate, filtered and the

with the Obermayer-Wang-Ellinger method and was much simpler and more rapid in execution.

The seventh and eighth columns in the tables headed "Number of defecations in number of days," and "Number of defecations in 3 days preceding analysis," represent an attempt to standardize the term "constipation." A patient was termed constipated if the number of his normal defecations was half or less than half the number of days between examinations. Of course, in some instances a patient would have several defecations in one day and none for a day or two following, in which case the character of the bowel movements was considered, and as long as they were soft and well formed the patient was not termed constipated. However, this information gave only a general idea of the condition during the intervals between analyses. Since these were often a week or ten days the question arose as

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clear filtrate mixed with an equal volume of Obermayer's reagent (Obermayer's reagent is prepared by adding 2 to 4 grams ferric chloride to one liter concentrated hydrochloric acid), and about 5 cc. chloroform. Upon shaking, the chloroform will assume a blue color if indican be present, the intensity of which will vary with the amount of indigo blue which has been brought into solution by the chloroform. Qualitatively the depth of color may be taken as indication of the degree of indicanuria. Normally only a faint blue color is produced.

b. Quantitative estimation: The quantity of urine depends upon the amount of indican present. Where qualitative tests have shown strong reactions for indican it will be found convenient to employ 35 to 50 cc. of urine. A little basic lead acetate is added to faintly acid urine and the mixture shaken, filtered, and two 35 to 50 cc. portions (depending on the qualitative test), are taken to serve as duplicates and treated in separatory funnels with equal volumes of Obermayer's reagent, and an accurately measured amount of chloroform (10 cc.). The mixture is then shaken and after separation of chloroform this is withdrawn, and fresh portions of measured amounts of chloroform added until the extracts become colorless. The extracts are then combined and filtered. The clear filtrate is compared in a Duboscq colorimeter with a standard solution of pure indigo blue in chloroform. The use of a chloroform solution of indigotin as standard is to be preferred to the use of a standardized Fehling's solution as recommended by Folin (American Journal of Physiology, 1905, *13*, p. 53). Meyers and Fine have found that disproportionate changes in the depth of an indigotin solution do not show the same proportionate changes in matching up with Fehling's solution. Further, when Fehling's solution is diluted one half with water and compared with a concentrated solution, it does not show double the colorimeter read-to the patient's condition immediately before collection of urine. A constipated state preceding the analysis might exist without being suspected from the total number of defecations for that period. Another tabulation was made, therefore, showing the number of defecations that had occurred during the three days preceding the analysis. Thus if an analysis is dated February 27th the collection of urine would have been begun February 26th and the number in the eighth column represents the number of bowel movements for February 24th, February 25th, and February 26th.

Of the twenty-five control cases four were extremely constipated one had diarrhea, and twenty enjoyed satisfactory daily defecations. In a sense these twenty served as normal controls, being, as far as bowel movements are concerned, entirely normal. The

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ing. The standard indigotin is prepared by adding an excess of Kahlbaum's pure indigotin to warm chloroform, allowing it to stand over night and then filtering to remove any suspended indigotin. The strength of this standard is determined by titrating the indigotin solution with approximately N/400 potassium permanganate according to the method of Ellinger (Zeitschrift für Physiologische Chemie, 1903, *38*, p. 178). The method is as follows: 10 cc. portions of the chloroform extract are evaporated to dryness in small extraction flasks, treated with 10 cc. of concentrated sulphuric acid and heated on the water bath for 5 minutes. They are then treated with 100 cc. of distilled water and titrated with the permanganate until the last trace of blue color disappears and only a pale yellow remains. The permanganate is prepared by taking 5 cc. of a solution of pure potassium permanganate (3.000 grams to 1 liter) and diluting to 200 cc. with water. One cubic centimeter of this solution is equivalent to 0.15 mg. indigotin. To obtain the value of the indigotin solution in terms of indican, multiply by 1.92. This solution will keep for a considerable length of time in a well stoppered amber bottle in the dark. The standard solution is set at 15 mm. in the Duboscq colorimeter. The calculation is as follows:

$$\frac{S}{\mathrm{R}} \times 0.23 \times \frac{F}{10} \times \frac{T.V.}{V.} = \text{milligrams indican}$$

in 24 hour specimen of urine

S = reading of standard which is 15 mm.
R = reading of unknown.
0.23 = indican present in 10 cc. of a saturated solution of indigotin.
F = volume of $CHCl_3$ filtrate.
10 = volume of $CHCl_3$ filtrate in Duboscq cup.
$T.V.$ = total volume of urine.
V = volume of urine used for analysis.

TABLE 62

Records of urinary examinations

Case 43. Female, age 25 years, dementia praecox

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	*cc.*		*mg.*						
L. acidophilus milk, February 28	February 28	1,170	Acid	—	10.77	E 3 in 7	1	..	..	..	...
	March 8	865	Acid	—	13.09	3 in 8	1	18	82	95	5
	March 16	1,265	202.0	16	5.50	E 4 in 8	s 2	26	74	90	10
	March 20	975	Acid	—	16.77	2S 2 in 4	s 1	20	80	60	40
	April 15	900	504.0	56	19.70	3E 18 in 26	1	..	..	..	...
	April 24	975	312.0	32	13.90	6 in 9	2	..	..	..	...
	May 14	825	330.0	40	19.50	4 in 7	2	25	75	0	100
L. acidophilus milk and lactose, May 23	June 3	725	348.0	48	10.92	S 9 in 20	3	40	60	20	80
	June 6	465	Alk.	Alk.	.....	....	..	..	..	..	..
	....	.....	223.6	48	1.17	2 in 3	2	..	..	..	..
	July 11	860	309.6	36	8.20	3S 21 in 35	1	85	15	0	100
	July 18	1,130	180.8	16	10.80	6 in 7	2	..	..	..	..
	July 24	1,250	900.0	72		4 in 6	2	32	68	0	100
	August 1	920	294.4	32	6.48	6 in 7	2	..	..	..	..

Legend for tables 62 to 109:

E = Defecation caused by enema during interval.
S = Defecation caused by suppository during interval.
e = Specific defecation caused by enema.
— = Not done.
* = Negative or slight trace.
Alk. = Alkaline.
Sl. alk. = Slightly alkaline.
Micro. = Microscopic

TABLE 63

Case 32. Female, age 42 years, dementia praecox

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
								Micro per cent		Plate per cent	
			N/10	Per cent				+	−	+	+
		cc.	cc.		mg.						
L. acidophilus milk and lactose, November 1, 1922	February 15	675	Acid	—	4.36	5 in 7	3	21	79	0	100
	March 8	690	Acid	—	1.14	7 in 7	3	30	70	2	98
	March 20	760	Acid	—	1.58	9 in 12	2	26	74	15	85
	March 28	925	143.0	16	12.30	2S 6 in 8	1	..	..	..	...
	April 12	1,765	70.6	4	*	2S 7 in 14	1	23	77	0	100
	April 23	1,260	Neutral	—	*	S 5 in 11	2	31	69	0	100
	April 30	875	70.0	8	*	3 in 7	2	..	..	..	...
	May 7	1,025	82.0	8	*	4 in 7	3	..	..	..	...
	May 16	2,275	91.0	4	5.55	8 in 9	3	31	69	95	5
	May 21	1,460	175.0	12	*	S 5 in 5	3	..	..	..	...
	May 28	1,730	415.0	24	11.95	5 in 5	3	31	69	0	100
	June 3	2,105	336.8	16	*	S 4 in 6	2	39	61	0	100
	June 6	1,245	448.0	36	*	3 in 3	3	..	..	..	...
	June 27	2,700	Alk.	Alk.	.....	....	..	..	..	..	...
	....	.....	540.0	20	*	S 18 in 21	3	18	82	0	100
	July 9	2,245	269.0	12	16.20	12 in 12	3	26	74	40	60
	July 17	3,150	252.0	8	*	8 in 8	3	28	72	75	25
	July 23	1,905	228.6	12	*	6 in 6	3	..	..	..	...
	July 30	2,365	283.8	12	*	9 in 7	4	..	..	..	...
No milk, 100 grams lactose August 3	August 6	1,700	544.0	32		8 in 6	5	27	73	0	100
	August 15	2,400	192.0	8	3.31	10 in 9	4	24	76	0	100

TABLE 64

Case 41. Female, age 42 years, dementia praecox

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
								Micro per cent		Plate per cent	
			N/10	Per cent				+	–	+	–
		cc.	cc.		mg.						
L. acidophilus paste, February 20	March 1	1,425	Acid	–	3.20	2 in 3	3	..	..	...	..
L. acidophilus milk, March 13	March 21	1,125	360.0	32	7.78	2E 14 in 20	0	13	87	100	0
	April 12	2,250	810.0	36	27.70	17 in 17	3	31	69	100	0
	April 15	2,680	859.0	32	*	3 in 3	3	..	..	...	..
	April 23	1,085	347.2	32	13.15	11 in 8	5	54	46	20	80
	April 30	1,495	598.0	40	31.50	9 in 7	4	27	73	95	5
	May 7	1,050	588.0	56	24.20	4 in 7	3	28	72	85	15
Treatment discontinued, May 23	June 6	900	288.0	32	6.84	6 in 6	3	30	70	50	50
	July 9	1,405	281.0	20	4.73	13 in 13	3	35	65	0	100
	July 16	1,475	472.0	32	*	7 in 7	3	42	58	0	100
	July 30	1,790	286.0	16		6 in 7	2	..	..	..	...

TABLE 65

Case 19. Female, age 37 years, dementia praecox

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	*cc.*		*mg.*						
L. acidophilus paste, March 28	April 12	1,300	312.0	24	*	2 in 5	0	. .	. .	. .	. . .
L. acidophilus milk, April 17	May 1	775	155.0	20	*	2S 5 in 18	0	28	72	0	100
	May 9	1,350	162.0	12	5.20	S 2 in 9	0	27	73	0	100
	May 16	1,230	393.6	32	4.72	S 2 in 7	0	20	80	0	100
L. acidophilus milk and lactose, May 23	June 3	2,690	322.8	12	*	2S 7 in 10	s 2	.	.	. .	. . .
	June 6	2,280	364.8	16	*	2 in 3	1	. .	. .	. .	. . .
	July 11	2,340	280.8	12	*	2S 18 in 14	3	28	72	0	100
	July 18	3,140	. . . . .	—	*	7 in 7	3	31	69	5	95
	July 23	2,875	345.0	12	*	5 in 5	3			.	. . .
	July 25	2,805	561.0	20	*	2 in 2	2			.	
	August 1	3,570	421.2	12	*	6 in 6	3				
	August 8	2,865	497.4	16	*	7 in 7	3				.
	August 15	3,410	409.2	12	*	6 in 7	3			.	. . .
Treatment discontinued, November 30	December 5	2,840	68.1	2.4	*	5 in 7	3			.	.

TABLE 66

Case 42. Female, age 25 years, dementia praecox

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
								Micro per cent		Plate per cent	
			N/10	Per cent				+	−	+	−
		cc.	*cc.*		*mg.*						
L. acidophilus milk, February 28	March 12	1,005	Acid	—	11.10	E 3 in 7	1	10	90	95	5
	March 21	1,625	Neutral	—	*	2E 0 in 9	e 1	13	87	95	5
	April 12	1,550	310.0	20	*	3E 22 in 23	3	30	70	95	5
	April 15	2,840	227.2	8	*	3 in 3	3	. .	. .	. .	. . .
	April 23	690	193.2	28	10.40	10 in 8	3	. .	. .	. .	. . .
	April 30	785	157.0	20	4.43	8 in 7	3	27	73	85	15
	May 9	1,100	—	—	*	S 11 in 9	6	. .	. .	. .	. . .
	May 16	630	147.2	24	5.02	9 in 7	4	22	78	90	10
	May 21	2,135	170.8	8	20.80	4 in 5	3	30	70	75	25
Treatment discontinued, May 23	May 28	1,925	453.0	24	10.60	7 in 7	3	31	69	0	100
	June 3	850	170.0	20	11.15	5 in 5	3	. .	. .	. .	. . .
	June 25	1,440	460.8	32	15.20	26 in 27	2	24	76	0	100
	July 9	1,930	617.6	32	16.25	S 10 in 14	3	25	75	0	100
	July 16	1,150	322.0	28	8.50	7 in 7	3	. .	. .	. .	. . .
	July 23	875	595.0	68	16.20	8 in 7	4	23	77	0	100
	July 30	1,150	276.0	24	11.37	10 in 7	4	23	77	0	100
	August 6	1,420	454.4	32	9.62	6 in 5	4	. .	. .	. .	. . .
	August 15	960	101.7	16	*	10 in 9	4	15	85	0	100

TABLE 67

Case 52. Male, age 20 years, dementia praecox

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	*cc.*		*mg.*						
Pasteurized *L. acidophilus* milk, January 30	February 28	976	Sl. alk.	—	17.89	9 in 7	6	70	30	0	100
	March 7	1,190	Neutral	—	9.75	5 in 7	2	26	74	0	100
	March 15	1,740	556.8	32	13.70	8 in 8	2	40	60	0	100
	March 22	800	128.0	16	5.40	5 in 7	0	38	62	0	100
L. acidophilus milk, March 27	March 27	610	195.2	32	7.25	2 in 5	0	23	77	99	1
	April 11	860	275.2	32	10.70	15 in 15	1	38	62	10	90
	April 18	850	374.0	44	23.60	6 in 7	4	26	74	95	5
	**April 24	1,840	588.8	32	10.49	8 in 6	3	38	62	80	20
	May 2	1,025	327.5	32	14.12	0 in 3	0	30	70	90	10
	May 9	910	327.6	36	13.15	4 in 7	2	..	..	..	...
	May 22	1,450	580.0	40	34.65	7 in 13	3	..	..	..	...
L. acidophilus milk and lactose, May 27	May 29	690	358.8	52	*	13 in 7	8	26	74	99	1
	June 5	875	560.0	64	9.70	9 in 7	4	..	..	..	..
	June 26	840	336.0	40	*	28 in 21	7	55	45	50	50
Treatment discontinued, June 28	July 3	1,455	407.2	28	4.75	8 in 7	2	27	73	0	100
	July 10	1,180	424.8	36	*	6 in 7	2	..	..	..	...
	†July 31	500	200.0	40	6.07	4 in 6	2	..	..	..	...
	August 7	1,245	697.2	56	*	5 in 7	2	13	87	0	100
	August 13	1,350	432.0	32	*	5 in 6	3	23	77	0	100

** Transferred April 24–30 for operation.

† Transferred August 11–24 to another ward.

TABLE 68

Case 58. Female, age 57 years, manic-depressive

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	cc.		mg.						
L. acidophilus filtrate, April 27	May 3	1,215	340.2	28	*	3 in 3	2	8	92	0	100
	May 7	1,100	396.0	36	6.15	6 in 4	5	. .	. .	. .	. . .
	May 14	1,585	634.0	40	8.20	10 in 7	4	30	70	0	100
L. acidophilus milk, May 23	May 23	750	405.0	54	6.30	10 in 9	4	24	76	0	100
	June 3	525	357.0	68	2.15	11 in 11	3	31	69	0	100
	June 6	410	264.0	64	3.27	3 in 3	3	. .	. .	. .	. . .
	June 27	450	243.0	54	7.55	17 in 21	2	41	59	0	100
Treatment discontinued, June 28	July 11	375	142.5	38	2.92	12 in 14	3	24	76	0	100
	July 25	500	160.0	32	1.54	16 in 14	3	27	73	0	100
	August 1	605	326.7	54	4.02	7 in 6	4	30	70	0	100
	August 8	575	370.8	72	2.62	9 in 7	3	30	70	5	95

TABLE 69

Case 33. Female, age 54 years, manic-depressive

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 5 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
								Micro per cent		Plate per cent	
			N/10	Per cent				+	–	+	–
		cc.	cc.		mg						
No treatment	February 28	400	Acid	–	*	0 in 1	0	. .	. .	. .	. . .
	March 5	435	Alk.	–	1.19	0 in 5	0	. .	. .	. .	. . .
	March 16	1,175	Neutral	–	*	1 in 9	1	. .	. .	. .	. . .
	March 19	625	100.0	16	1.88	0 in 3	0	. .	. .	. .	. . .
	March 21	1,550	248.0	16	*	1 in 2	1	. .	. .	. .	. . .
L. acidophilus paste, March 25	March 28	1,275	102.0	8	*	1 in 7	1	. .	. .	. .	. . .
	April 11	705	—	—	*	1 in 14	0	. .	. .	. .	. . .
	April 15	2,260	80.4	4	*	1 in 4	0	. .	. .	. .	. . .
L. acidophilus milk, April 17	April 24	1,680	403.0	24	5.69	1 in 9	0	. .	. .	. .	. . .
	May 1	2,270	363.2	16	7.60	1 in 6	0	. .	. .	. .	. . .
	May 9	1,610	322.0	20	*	1 in 8	0	26	74	0	100
	May 14	990	396.0	40	*	1 in 5	0				
	May 23	1,570	439.6	28	6.35	1 in 9	0	11	89	0	100
	June 3	1,140	456.0	40	*	3 in 11	1				
	June 6	405	291.6	72	*	0 in 3	0	35	65	0	100
	June 27	615	369.0	60	4.10	3 in 21	0	. .	. .	. .	. . .
L. acidophilus milk and lactose, June 28	July 11	550	242.0	44	4.30	4 in 14	0	85	15	80	20
	July 18	515	205.0	40	7.20	3 in 7	0	. .	. .	. .	. .
	July 23	680	326.4	48	19.15	1 in 5	0	. .	. .	. .	. .
	July 25	745	238.0	32	*	1 in 2	1	. .	. .	. .	. .
	August 1	1,205	289.2	24	6.05	1 in 6	0	. .	. .	. .	. .
	August 8	550	88.0	16	3.98	2 in 7	1	. .	. .	. .	. . .
	August 15	980	313.0	32	*	2 in 7	1	. .	. .	. .	. . .
	December 5	1,680	217.6	32	*						

TABLE 70

Case 46. Female, age 50 years, manic-depressive

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	cc.		mg.						
L. acidophilus filtrate, March 6	March 29	2,496	99.8	4	*	0 in 7	0	22	78	0	100
	April 12	975	234.0	24	*	1 in 15	0	33	67	0	100
	April 15	1,300	364.0	28	*	S 2 in 3	2	..	..	..	...
L. acidophilus milk, April 18	April 24	660	211.4	32	*	4 in 9	2	16	84	0	100
	May 1	1,275	102.0	8	*	2 in 6	1	34	66	0	
	May 9	440	70.4	16	*	S 2 in 9	1	..	..	..	...
	May 14	1,175	282.0	24	*	S 1 in 5	0	16	84	70	30
	May 23	1,605	64.2	4	6.05	1 in 9	1	33	67	0	100
	June 3	820	328.0	40	2.36	S 2 in 11	1	..	..	..	...
	June 6	1,560	187.2	12	8.30	1 in 3	1	..	..	..	...
	June 21	575	184.0	32	*	2E 4 in 21	0	..	..	..	...
L. acidophilus milk and lactose, June 28	July 11	875	210.0	24	*	3E 4 in 19	0	39	61	0	100
	July 24	335	67.0	20	7.05	4E 7 in 13	1	40	60	0	100
	August 1	1,380	220.8	16	*	2E 2 in 7	0	..	..	..	...
	August 8	705	394.8	56	*	E 2 in 7	e 1	85	15	0	100
	December 5	680	217.6	32	*	....	..	..	..	..	...

TABLE 71

Case 31. Female, age 61 years, manic-depressive

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	cc.		mg.						
No treatment	March 8	900	Acid	—	*	1 in 9	1				
	March 20	300	Acid	—	4.62	E 2 in 12	0	..	..	..	...
L. acidophilus milk, March 22	March 23	675	243.0	36	2.42	E 1 in 3	e 1	19	81	0	100
	March 28	545	130.8	24	4.42	E 2 in 5	0	..	..	..	...
	April 11	610	146.4	24	5.62	S 4 in 14	1	11	89	0	100
	April 12	390	140.0	36	3.80	0 in 1	1	..	..	..	...
	April 15	620	173.6	28	*	2E 2 in 3	2e 2	..	..	..	...
	April 23	720	115.2	16	*	3 in 8	2	40	60	0	100
	April 30	1,410	169.2	12	14.40	4 in 7	2	29	71	0	100
	May 7	1,035	248.4	24	19.30	4 in 7	2	31	69	0	100
	May 16	1,405	56.2	4	14.20	S 5 in 9	2	21	79	2	98
	May 21	1,115	223.0	20	7.82	S 5 in 5	2	31	69	0	100
L. acidophilus milk and lactose, May 23	May 28	1,435	344.4	24	13.45	4 in 7	2	30	70	0	100
	June 6	410	164.0	40	8.54	E 6 in 9	3	44	56	30	70
	June 25	785	188.4	24	7.05	3E 7 in 19	1	..	..	..	...
	July 9	1,480	232.4	16	*	4 in 14	0	..	..	..	...
	July 16	925	185.0	20	4.15	2E 5 in 7	2	..	..	..	...
	July 23	1,030	659.2	64	15.45	2E 4 in 7	2	85	15	15	85
	July 30	1,190	476.0	40	10.10	2E 3 in 7	1	..	..	..	...
	August 6	1,220	331.6	28	16.92	2E 4 in 7	2	33	67	0	100
	August 15	1,495	538.2	36	8.57	3E 6 in 9	e 3	85	15	40	60
	1924										
Treatment discontinued	April 4	2,300	230.0	10	.....	....	...	..	..	..	...
	April 7	2,900	185.6	6	.....	....	...	..	..	..	...
	April 9	2,300	184.0	8	.....	....	...	..	..	..	...

TABLE 72

Case 29. Female, age 38 years, manic-depressive

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	cc.		mg.						
L. acidophilus filtrate, February 14	February 20	1,710	Acid	—	*	5 in 7	2	..	..	..	...
	February 28	1,970	Acid	—	*	1 in 8	1	23	77	0	100
	March 5	2,325	Acid	—	1.48	2 in 5	1	..	..	..	...
	March 12	2,820	Neutral	—	4.29	E 6 in 7	e 2	33	67	0	100
L. acidophilus milk, March 13	March 20	2,080	Acid	—	*	4 in 8	4	26	74	0	100
	March 26	3,975	477.0	12	*	3 in 6	3	...	..	..	...
	March 29	2,975	238.0	8	6.65	1 in 3	1	21	79	0	100
	April 12	2,035	488.4	24	*	13 in 14	3	23	77	0	100
	April 15	1,935	464.4	24	*	3 in 3	3	..	..	..	...
	April 16	1,395	167.4	12	*	3 in 3	3	..	..	..	...
	June 26	915	219.6	24	*	61 in 71	2	38	62	0	100
Treatment discontinued, June 28	July 9	2,020	161.6	8	*	15 in 13	3	21	79	0	100
	July 16	2,750	Alk.	Alk.	....	......	..	..	..	..	...
			220.0	8	*	6 in 6	3	..	..	..	...
	July 17	1,550	268.0	16	*	1 in 1	3	..	..	..	...
	July 23	2,550	408.0	16	*	6 in 6	3	..	..	..	...
	July 25	2,280	182.4	8	*	2 in 2	3	..	..	..	...
	July 30	3,015	482.5	16	*	5 in 5	3	..	..	..	...
	August 6	1,765	423.6	24	*	8 in 7	3	..	..	..	...
	August 15	1,745	289.2	16	*	8 in 9	2	27	73	0	100
	August 20	2,285	584.4	24	*	5 in 5	3	..	..	..	...
	April 4, 1924	1,600	128.0	8	....	......	..	..	..	..	...

TABLE 73

Case 30. Female, age 27 years, manic-depressive

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	cc.		mg.						
No treatment...............	February 21	2,825	Acid	—	7.71	6 in 7	3	..	..	.	..
	February 28	1,300	Acid	—	2.37	7 in 7	3	..	..	..	..
L. acidophilus milk and lactose, March 5	March 8	2,050	Acid	—	7.25	7 in 8	3	..	..	..	..
	March 14	1,375	990.0	72	9.50	child birth no record	..	..	..	..	..
	June 27	875	266.0	56	3.10	13 in 14	2	..	..	..	..
	July 11	750	90.0	12	8.40	14 in 14	3	..	..	..	..
	July 23	965	308.0	32	7.32	12 in 12	3	41	59	0	100

TABLE 74

Case 37. Female, age 21 years, manic-depressive

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
								Micro per cent		Plate per cent	
			N/10	Per cent				+	−	+	−
		cc.	cc.		mg.						
L. acidophilus milk, March 12	March 12	1,015	Acid	—	7.82	10 in 12	5	36	64	0	100
	March 29	2,118	338.8	16	14.00	0 in 3	0	27	73	0	100
	April 11	1,675	268.0	16	10.19	S 4 in 13	1	18	82	0	100
	April 23	1,080	432.0	50	10.03	S 6 in 12	4	..	..	..	...
	April 30	900	432.0	48	28.30	3 in 7	0	..	..	..	...
	May 7	1,615	387.6	24	14.70	S 2 in 7	1	38	62	25	75
	May 9	2,200	440.0	20	11.58	1 in 2	1	29	71	35	65
	May 16	2,905	697.2	24	20.84	2 in 7	1	30	70	50	50
	May 21	2,935	117.4	4	*	3 in 5	2	85	15	10	90

TABLE 75

Case 38. Female, age 18 years, manic-depressive

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	cc.		mg.						
L. acidophilus milk.........	February 15	530	Acid	—	2.82	7 in 7	5	28	72	100	0
	February 20	795	Acid	—	3.62	4 in 5	2	..	..	..	...
Treatment discontinued, April 6	April 23	1,525	488.0	32	11.35	28 in 17	8	47	53	0	100
	April 30	2,420	580.8	24	46.20	24 in 7	9	13	87	0	100

TABLE 76

Case 68. Female, 38 years, manic-depressive

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
								Micro per cent		Plate per cent	
			N/10	Per cent				+	−	+	−
		cc.	cc.		mg.						
No treatment.............	March 1	1,375	Acid	—	*	....	..	..	..	..	...
	March 21	2,450	392.0	16	5.95	....	..	..	..	..	...
	March 28	1,225	245.0	20	5.15	....	..	..	.	..	...
	April 11	3,625	290.0	8	*	4 in 17	1	12	88	0	100
	April 15	4,085	163.4	4	*	S 1 in 4	0	31	69	0	100
	April 24	2,920	232.0	8	*	8 in 9	4	..	..	..	...
L. acidophilus filtrate, April 27	May 1	3,025	121.0	4	*	1 in 6	0	25	75	0	100
	May 10	2,415	96.6	4	*	S 5 in 10	0	..	..	..	...
	May 16	2,710	216.8	8	*	S 3 in 6	1	40	60	0	100
Treatment discontinued, May 17	May 24	2,690	215.2	8	8.77	5 in 8	2	30	70	0	100
	June 3	3,810	762.0	20	8.37	S 4 in 10	1	..	..	..	...
	June 6	3,975	954.0	24	*	3 in 6	3	39	61	0	100
	June 27	2,700	864.0	32	*	11 in 21	1	22	78	0	100
	July 18	4,275	342.0	8	*	5 in 6	2	21	79	0	100
	July 25	3,500	280.0	8	*	5 in 7	2	..	..	..	...
	August 1	4,095	491.4	12	*	6 in 6	3	40	60	0	100
	August 8	3,730	298.4	8	*	7 in 7	3	..	..	..	...
	August 15	3,510	372.0	10.6	*	6 in 7	3	38	62	0	100

TABLE 77

Case 50. Male, age 30 years, manic-depressive

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	cc.		mg.						
Pasteurized *L. acidophilus* milk, January 30	March 7	790	Neutral	—	*	2 in 7	1 in 3	30	70	0	100
	March 15	1,160	Neutral	—	7.75	3 in 8	2 in 3	35	65	0	100
	March 22	1,900	152.0	8	9.14	0 in 7	0 in 3	38	62	0	100
L. acidophilus filtrate, March 26	March 27	1,660	199.0	12	20.00	1 in 5	0 in 3	20	80	0	100
	April 11	2,310	369.6	16	21.18	5 in 15	1 in 3	27	73	0	100
L. acidophilus milk, April 17	April 18	1,010	202.0	20	15.40	4 in 7	2 in 3	29	71	100	0
	April 24	1,590	318.0	20	14.88	4 in 6	1 in 3	39	61	50	50
	May 2	675	270.0	40	12.90	5 in 8	1 in 3	26	74	30	70
	May 9	1,720	825.0	48	11.10	3 in 7	2 in 3	41	59	10	90
	May 10	650	312.0	48	9.63	1 in 1	2 in 3				
	May 22	1,250	250.0	20	6.00	4 in 12	1 in 3	47	53	0	100
L. acidophilus milk and lactose, May 27	May 29	1,100	264.0	24	5.72	2 in 7	1 in 3	40	60	2	98
	June 5	890	499.0	56	7.89	4 in 7	2 in 3	..	..	..	...
	June 26	475	310.0	64	5.37	7 in 21	1 in 3	30	70	50	50
	July 3	1,275	153.0	12	7.50	2 in 7	1 in 3				..
	July 10	635	279.4	44	10.32	4 in 7	2 in 3			.	.
	July 17	425	211.0	52	16.40	3 in 7	2 in 3			..	.
	July 31	1,200	288.0	24	7.03	5 in 9	2 in 3	.	..	..	..
	August 7	1,225	392.0	32	27.20	1 in 7	0 in 3	..	..	..	..
	August 13	600	384.0	64	10.90	5 in 6	1 in 3	..	..	20	80
	August 21	1,025	328.0	32	16.30	6 in 8	1 in 3	..	..	..	...
	1924										
Treatment discontinued....	April 4	1,690	277.2	16	.....	......	......	..	..	..	...
	April 7	1,020	314.1	30	.....	......	......	..	..	..	...

TABLE 78

Case 53. Male, age 39 years, manic-depressive

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	–	+	–
		cc.	cc.		mg.						
Pasteurized *L. acidophilus* milk, January 30	February 27	1,655	Neutral	–	5.23	3 in 7	1	45	55	0	100
	March 6	900	Neutral	–	4.97	4 in 7	1	19	81	0	100
	March 15	1,000	Neutral	–	3.60	7 in 9	3	32	68	0	100
	March 22	1,960	Neutral	–	19.05	7 in 7	5	31	69	0	100
L. acidophilus paste, March 26	March 27	2,490	398.0	16	14.00	1 in 5	1	18	82	50	50
	March 29	1,550	435.0	28	15.00	1 in 2	1	..	..	..	...
	April 11	2,230	624.4	28	10.15	7 in 13	1	18	82	0	100
L. acidophilus milk, April 17	April 18	1,350	324.0	24	29.60	3 in 7	1	13	87	40	60
	April 24	1,550	310.0	20	*	5 in 6	5	27	73	40	60
	May 2	1,675	344.0	20	9.70	5 in 8	0	23	77	25	75
	May 9	1,295	363.0	28	8.26	4 in 7	1	48	52	90	10
	May 10	1,675	602.0	36	9.68	0 in 1	0	..	..	..	...
	May 22	1,725	552.0	32	13.68	8 in 12	3	27	73	99	1
L. acidophilus milk and lactose, May 27	June 5	645	464.0	72	*	16 in 7	11	..	..	..	...
	June 26	1,175	658.0	56	*	53 in 21	3	..	..	10	90
Treatment discontinued, June 28	July 3	1,200	576.0	48	6.50	10 in 7	4	21	79	0	100
	July 10	1,190	541.2	48	17.50	6 in 7	3	..	..	..	...
	July 17	1,200	336.0	28	11.35	10 in 7	4	..	..	..	...
	July 22	870	487.2	56	2.73	8 in 5	5	47	53	0	100
	July 31	1,000	560.0	56	5.00	10 in 9	2	19	81	0	100
	August 7	1,205	482.0	40	*	8 in 7	3	..	..	..	...
	August 13	1,000	320.0	32	*	9 in 6	4	24	76	0	100

TABLE 79

Case 54. Male, age 31 years, manic-depressive

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	cc.		mg.						
Pasteurized *L. acidophilus* milk, January 30	March 6	1,250	Acid	—	8.65	5 in 7	3	. .	. .	0	100
	March 15	1,750	420.0	24	8.69	9 in 9	3	36	64	0	100
	March 22	1,320	316.8	24	10.27	6 in 7	2	27	73	0	100
L. acidophilus paste, March 26	March 27	1,665	666.0	40	10.70	2 in 5	1	. .	. .	. .	. . .
	March 29	1,720	963.2	56	27.20	1 in 2	1	20	80	90	10
	April 11	1,420	738.4	52	10.20	7 in 13	0	23	77	0	100
L. acidophilus milk, April 17	April 18	2,500	1,200.0	48	16.50	6 in 7	3	19	81	99	1
	April 24	1,000	480.0	48	13.50	4 in 6	2	28	72	0	100
	May 2	850	374.0	44	13.73	4 in 8	2	28	72	20	80
	May 9	1,325	689.0	52	13.10	4 in 7	2	31	69	60	40
	May 10	705	366.6	52	11.45	1 in 1	2	. .	. .	. .	. . .
	May 22	1,430	743.6	52	18.65	6 in 12	1	19	81	50	50
L. acidophilus milk and lactose, May 27	May 29	480	249.6	52	7.86	7 in 7	2	40	60	60	40
	June 5	810	550.8	68	9.25	4 in 7	3				
	June 26	600	480.0	80	4.50	9 in 21	0				
	July 3	1,525	488.0	32	11.50	4 in 7	0				
	July 10	750	450.0	60	13.50	5 in 7	0		. .	. .	.

L. acidophilus milk and lactose, May 27	July 17	1,325	583.0	44	12.95	2 in 7	1	..	..	..	...
	July 22	1,000	520.0	52	*	9 in 5	7	..	..	..	...
	July 31	1,250	500.0	40	2.30	2 in 9	1	..	..	..	...
	August 7	925	444.0	48	10.91	1 in 7	0	27	73	0	100
	August 13	625	400.0	64	*	4 in 5	0	50	50	..	...
	August 21	850	408.0	48	*	4 in 8	0	..	..	..	...
	1924										
Treatment discontinued....	April 4	900	388.8	43	.....	......	..	..	..	..	...
	April 7	860	392.1	45	.....	......	..	..	..	..	...
	April 8	1,080	518.4	48	.....	......	..	..	..	..	...

TABLE 80

Case 51. Male, age 20 years, psychoneurosis

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	cc.		mg.						
Pasteurized *L. acidophilus* milk, January 30	February 27	2,809	Neutral	—	*	7 in 7	2	..			...
	March 7	1,840	Neutral	—	4.28	10 in 8	4	33	67	0	100
	March 15	1,510	Acid	—	*	8 in 8	2	19	81	0	100
	March 22	1,450	174.0	12	*	6 in 7	3	22	78	0	100
L. acidophilus filtrate, March 26	March 27	2,110	84.4	4	8.64	5 in 5	2	34	66	0	100
	April 11	2,230	267.6	12	10.15	12 in 15	3	39	61	0	100
L. acidophilus milk, April 17	April 18	1,580	568.8	36	27.30	7 in 7	2	23	77	98	2
	May 10	1,955	156.4	8	5.20	24 in 22	1	50	50	95	5
	May 22	2,200	264.0	12	23.20	10 in 12	1	40	60	0	100
L. acidophilus milk and lactose, May 27	May 29	1,355	542.0	40	*	8 in 7	5	85	15	50	50
	June 5	1,000	320.0	32	*	8 in 7	4	..	.	..	...
	June 26	1,935	464.4	24	4.98	30 in 21	5	38	62	95	5
Treatment discontinued, June 28	July 3	2,250	90.0	4	*	7 in 7	3	26	74	0	100
	July 10	1,990	318.0	16	*	7 in 7	3	..	..	..	...
	July 17	2,014	322.2	16	*	6 in 7	4	..	.	..	...
	July 22	2,525	202.0	8	*	1 in 5	1	.	.	..	...
	July 31	2,470	197.0	8	*	4 in 9	2	..	..	..	...
	August 7	2,055	411.0	20	*	3 in 7	1	..	.	..	...
	August 13	1,935	464.4	24	*	4 in 6	1	45	55	0	100
	August 21	2,135	427.0	20	*	5 in 8	2	40	60	0	100
	1924										
	April 4	1,900	288.8	15	.....	.....	..	..	..	..	...

TABLE 81

Case 49. Female, age 35 years, psychoneurosis

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	*cc.*		*mg.*						
L. acidophilus filtrate, April 27	May 1	1,090	261.6	24	2.95	0 in 4	0	30	70	0	100
	May 10	810	.....	—	*	12 in 9	5	30	70	0	100
	May 14	1,125	315.0	28	*	3 in 4	2	25	75	0	100
L. acidophilus milk, May 23	June 3	1,025	287.0	28	23.80	15 in 19	3	34	66	65	35
	June 27	545	109.0	20	*	16 in 24	1	23	77	0	100
L. acidophilus milk and lactose, June 23	July 11	575	115.0	20	7.30	25 in 14	4	..	..	..	...
	July 18	675	216.0	32	*	8 in 7	4	85	15	20	80
	July 25	865	232.2	28	*	8 in 7	4	..	..	..	...
	August 1	1,525	366.0	24	3.56	7 in 7	4	63	37	0	100
	August 8	1,215	486.0	40	*	8 in 7	3	85	15	20	80

TABLE 82

Case 48. Female, age 26 years, psychoneurosis

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	cc.		mg.						
L. acidophilus filtrate, April 27	May 1	550	198.0	36	*	0 in 4	0				
	May 16	955	382.0	40	*	S 1 in 16	0	17	83	0	100
L. acidophilus milk, May 23	May 23	285	68.0	24	*	1 in 7	0	...	...	...	...
	June 6	500	200.0	40	*	2 in 13	0	...	...	...	...
L. acidophilus milk and lactose, July 6	July 11	750	150.0	20	*	8 in 35	0	85	15	10	90
	July 25	465	93.0	20	*	11 in 14	3	85	15	0	100
	August 1	275	11.0	4	*	3 in 6	0	65	35	0	100

TABLE 83

Case 44. Female, age 51 years, involutional melancholia

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	*cc.*		*mg.*						
L. acidophilus paste, February 26	February 28	490	Sl. alk.	—	*	1 in 1	. .	. .	. .	. .	. . .
	March 5	475	Acid	—	1.07	0 in 5	0	. .	. .	. .	. . .
	March 12	600	Acid	—	*	0 in 7	0	. .	. .	. .	. . .
	March 19	670	321.0	48	2.86	S 1 in 7	0	. .	. .	. .	. . .
	March 28	400	32.0	8	2.17	S 1 in 9	0	42	58	5	95
L. acidophilus filtrate, April 12	April 12	670	160.8	24	6.62	2S 2 in 13	1	33	67	0	100
	April 15	490	254.8	52	9.14	S 0 in 4	1	. .	. .	. .	. . .
L. acidophilus milk, April 17	April 24	600	264.0	44	7.69	5 in 9	3	. .	. .	. .	. . .
	May 1	535	321.6	60	12.90	2 in 6	1	. .	. .	. .	. . .
	May 14	575	184.0	32	8.37	S 1 in 4	1	14	86	0	100
	May 23	430	68.8	16	4.83	S 4 in 9	2	. .	. .	0	100
	June 6	705	338.4	48	2.54	8 in 14	3	16	84	40	60
	June 27	695	278.0	40	6.10	S 12 in 21	2	. .	. .	. .	. . .
Treatment discontinued, June 28	July 11	875	105.0	12	*	S 14 in 14	3	33	67	0	100
	July 18	625	250.0	40	3.70	6 in 7	3	27	73	0	100
	July 25	480	153.6	32	5.20	7 in 7	3	. .	. .	. .	. . .
	August 1	360	216.0	60	2.39	7 in 7	3	35	65	0	100
	August 8	230	220.8	96	2.38	8 in 7	3	. .	. .	. .	. . .
	August 15	625	200.0	32	*	5 in 7	3	. .	. .	. .	. . .

TABLE 84

Case 47. Female, age 64 years, alcoholic paranoia

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	cc.		mg.						
L. acidophilus paste, April 27	May 1	440	35.2	8	*	1 in 3	0	27	73	0	100
	May 9	575	46.0	8	*	5 in 9	1	..	..	..	...
	May 16	600	144.0	24	3.60	2 in 7	1	..	..	..	...
L. acidophilus milk, May 23	May 24	885	35.4	4	5.59	6 in 8	2	27	73	20	80
	June 3	380	152.0	40	*	10 in 9	3	23	77	0	100
	June 27	615	123.0	20	2.59	17 in 24	2	30	70	0	100
L. acidophilus milk and lactose, June 28	July 18	1,475	115.6	8	*	16 in 21	3	27	73	0	100
	July 25	1,320	105.6	8	4.97	7 in 7	3	..	..	..	...
	August 1	595	190.4	32	3.06	4 in 6	2	26	74	0	100
	August 8	935	374.0	40	4.80	5 in 7	2	35	65	50	50
	1924										
Treatment discontinued....	April 4	860	17.2	2	....	......	..	..	..	..	...
	April 7	1,400	98.0	7	....	......	..	..	..	..	...
	April 9	1,060	133.5	13	....	......	..	..	..	..	...

TABLE 85

Case 16. Female, age 25 years, dementia praecox. Control case

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	cc.		mg.						
No treatment.............	March 12	1,185	Neutral	—	7.89	2 in 4	1	33	67	0	100
	March 19	1,885	529.8	28	*	4 in 7	3	..	..	..	...
	March 26	1,130	406.5	36	12.80	S 4 in 7	1	26	64	0	100
	April 11	1,625	465.0	28	*	2S 8 in 14	2	21	79	0	100
	April 23	1,135	Neutral	—	3.10	S 9 in 12	2	23	77	0	100
	April 30	1,675	134.0	8	*	4 in 7	1	40	60	0	100
	May 7	1,000	160.0	16	*	4 in 7	3	23	77	0	100
	May 16	1,225	490.0	40	4.45	5 in 9	1	20	80	10	90
	May 21	1,200	240.0	20	8.10	3 in 5	2	28	72	0	100
	June 25	1,415	41.5	4	*	23 in 35	2	38	62	0	100
	July 9	1,405	224.8	16	4.15	7 in 14	1	21	79	0	100
	August 20	1,450	348.0	24	10.22	August 5–20 14 in 15	3	27	73	0	100

TABLE 86

Case 40. Female, age 28 years, dementia praecox. Control case

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
			N/10	Per cent				Micro per cent		Plate per cent	
								+	−	+	−
		cc.	cc.		mg.						
No treatment..........	February 27	1,035	Acid	—	*	2 in 7	0	40	60	0	100
	March 8	1,735	Acid	—	*	2 in 9	1	28	72	0	100
	March 20	1,600	Acid	—	*	7 in 12	1	32	68	5	95
	March 26	2,525	303.0	12	*	4 in 6	2	33	67	0	100
	April 11	1,900	228.0	12	*	April 1–11 5 in 11	1	16	84	0	100
	May 21	1,995	71.0	4	*	May 11–21 1 in 10	0	..	..	..	...
	May 23	1,205	96.0	8	*	0 in 2	0	..	..	..	...
	May 28	1,185	94.8	8	*	3 in 5	2	32	68	0	100
	June 6	775	62.0	8	*	4 in 9	2	45	55	0	100
	July 9	2,215	117.2	8	*	2 in 9	2	..	..	..	...
	July 16	800	32.0	4	*	2 in 7	2	..	..	..	...

TABLE 87

Case 69. Female, age 23 years, dementia praecox. Control case

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS
			N/10	Per cent			
		cc.	cc.		mg.		
No treatment..	March 21	2,075	Neutral	—	*	14 in 20	1
	March 28	1,800	72.0	4	*	6 in 7	2
	April 11	1,375	220.0	16	*	12 in 14	3
	April 16	2,485	99.4	4	*	5 in 5	3
	April 23	1,145	137.4	12	*	7 in 7	2
	May 1	2,225	89.0	4	*	9 in 7	6

TABLE 88

Case 70. Female, age 28 years, dementia praecox. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	cc.		mg.
No treatment....	June 3	1,660	158.4	9	20.30
	June 26	980	548.8	56	12.80
	July 9	2,430	388.8	16	*
	July 16	1,475	413.0	28	*
	July 23	680	272.0	40	3.52
	July 30	920	441.6	48	6.95
	August 8	580	139.2	24	*

TABLE 89

Case 71. Female, age 25 years, dementia praecox. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	cc.		mg.
No treatment.....	April 16	975	234.0	24	6.13

TABLE 90

Case 72. Female, age 22 years, dementia praecox. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	cc.		mg.
No treatment....	March 28	1,350	108.0	8	*
	April 16	350	98.0	28	*
	April 30	500	280.0	56	*
	May 7	550	176.0	32	*
	May 21	400	128.0	32	2.89
	May 28	600	312.0	52	*
	June 3	900	144.0	16	*
	June 25	675	81.0	12	*
	July 9	485	271.6	56	*
	July 16	700	280.0	40	2.67

TABLE 91

Case 73. Male, age 17 years, dementia praecox. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	cc.		mg.
No treatment....	March 8	980	Acid	–	8.73
	May 2	650	208.0	32	*
	May 9	715	.....	–	2.38

TABLE 92

Case 74. Male, age 20 years, dementia praecox. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	cc.		mg.
No treatment....	March 14	1,280	409.7	32	*
	March 23	810	324.0	40	1.69

patients receiving treatment were for the most part severely constipated.

Diagnoses of the mental conditions of the patients were as follows: In the control group: twelve dementia praecox, seven manic-depressive, five general paresis, and one psychoneurosis; in the group receiving treatment: six dementia praecox, twelve manic-depressive, three psychoneurosis, one involutional melancholia, and one alcoholic paranoia.

TABLE 93

Case 75. Male, age 23 years, dementia praecox. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	*cc.*		*mg.*
No treatment....	March 14	1,010	323.0	32	9.70
	March 23	930	287.0	32	7.80
	April 24	950	275.0	28	12.60
	May 2	1,225	196.0	16	10.35
	May 10	1,375	110.0	8	17.50
	May 22	1,710	205.2	12	*
	June 26	1,035	124.2	12	7.10
	July 3	762	30.4	4	3.95
	July 10	1,177	282.4	24	13.50

TABLE 94

Case 76. Male, age 25 years, dementia praecox. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	*cc.*		*mg.*
No treatment....	March 15	900	360.0	40	6.20
	March 22	980	470.4	48	12.41
	March 27	925	296.0	32	7.58
	April 18	830	199.2	24	15.50
	May 2	1,380	276.0	20	7.29
	May 10	600	168.0	28	4.15
	May 22	995	318.4	32	10.78

An examination of these tables 62 to 109 reveals some interesting facts. The volume of urine seems at first sight to be more variable than one would expect; but when one considers that these patients are psychotic this is not surprising. At first it was suspected that the urines might not represent the complete twenty-four hour elimination and many collections were therefore discarded. Upon inquiry and observation of the patients, however, it was frequently found that when a low volume of urine was obtained, the patient had had little fluid previous to and during the collection. Many of these patients must be forced to drink and their fluid intake is therefore very variable from day to day. Hawk (74) has reported that the volume of urine excreted during any twenty-four hour period is not necessarily proportionate to the amount of liquid ingested, and Folin (64) suggests that this may be due to variable amounts of water being excreted through the lungs or skin. How much more true is this of psychotic patients whose skin condition may in a short time range from excessive dryness to continual perspiration? Extreme care was taken to ensure complete twenty-four

TABLE 95

Case 77. Male, age 32 years, dementia praecox. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	cc.		mg.
No treatment....	March 8	2,400	Sl. alk.	–	
	April 18	3,190	Sl. alk.	–	7.03
	May 9	2,500	....	–	4.14
	May 22	1,750	70.2	4	6.96
	May 29	1,315	52.4	4	3.45
	June 5	2,428	96.8	4	*
	June 26	1,950	624.0	32	1.15
	July 3	2,725	108.8	4	*
	July 10	3,500	140.0	4	*
	July 17	2,675	213.6	8	4.35

TABLE 96

Case 78. Male, age 24 years, dementia praecox. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	cc.		mg.
No treatment....	March 8	850	Acid	–	7.34
	April 18	550	Acid	–	2.40
	May 2	725	174.0	24	*

TABLE 97

Case 18. Female, age 43 years, manic-depressive. Control case

TREATMENT	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN	NUMBER OF DEFECATIONS IN NUMBER OF DAYS SINCE LAST ANALYSIS	NUMBER OF DEFECATIONS IN 3 DAYS PRECEDING ANALYSIS	BACTERIAL COUNT			
								Micro per cent		Plate per cent	
			N/10	Per cent				+	−	+	−
		cc.	cc.		mg.						
No treatment...............	April 12	1,000	80.0	8	*	0 in 7	0	13	87	0	100
	April 15	2,100	252.0	12	*	0 in 3	0	...	..	..	...
	April 23	2,300	184.0	8	*	2E 2 in 8	e 1	25	75	0	100
	April 30	1,745	139.6	8	18.8	S 2 in 7	0	..	..	..	...
	May 7	2,050	205.0	8	*	E 2 in 7	1	29	71	0	100
	May 9	950	76.0	8	*	1 in 2	1	20	80	0	100
	May 21	2,375	95.0	4	*	2 in 7	0	17	83	10	90
	May 28	1,050	168.0	16	2.97	3 in 7	1	37	63	0	100
	June 6	1,690	540.8	32	*	4 in 9	2	..	..	..	...
	June 25	1,880	601.6	32	*	10 in 19	0	32	68	..	...
	July 9	1,375	495.0	36	*	10 in 14	3	..	..	..	...
	July 16	1,025	246.0	24	2.95	5 in 7	3	..	..	..	...
	July 30	2,510	602.4	24	*	6 in 7	3	..	..	..	...
	August 6	1,540	369.6	24	*	5 in 7	2	29	71	0	100
	August 15	1,775	.....	—	*	9 in 9	3	31	69	0	100
	August 20	2,445	195.6	8	*	5 in 5	3		..	..	...

TABLE 98

Case 35. Female, age 25 years, manic-depressive. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	cc.		mg.
No treatment	February 15	1,375	Acid		5.20

TABLE 99

Case 79. Female, age 45 years, manic-depressive. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	cc.		mg.
No treatment	April 16	1,546	556.5	36	*
	April 23	1,625	390.0	24	3.12
	May 1	1,565	375.0	24	*

TABLE 100

Case 80. Female, age 26 years, manic-depressive. Control case

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	cc.		mg.
No treatment	February 20	545	Acid	—	*
	April 24	955	305.6	32	*
	April 30	1,345	534.0	40	10.80
	May 7	970	77.6	8	*
	May 21	1,050	462.0	44	8.05
	May 28	700	420.0	60	3.14
	June 6	650	26.0	4	*
	July 16	1,580	632.0	4	*

TABLE 101

Case 81. Male, age 53 years, manic-depressive. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	cc.		mg.
No treatment	March 14	1,350	Acid	—	*
	March 23	1,750	559.0	32	*

TABLE 102

Case 82. Male, age 23 years, manic-depressive. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	cc.		mg.
No treatment....	March 14	1,120	560	50	*
	March 23	820	...	—	3.72

TABLE 103

Case 83. Male, age 16 years, psychoneurosis. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	cc.		mg.
No treatment....	March 14	1,420	454.4	32	*
	March 23	1,350	108.0	8	*
	May 9	1,100	.....	—	4.27
	May 22	1,500	360.0	24	8.55
	May 29	1,600	128.0	8	3.61
	June 5	1,000	640.0	64	4.85
	June 26	1,150	276.0	24	*
	July 3	1,175	188.0	16	*
	July 10	1,925	153.6	8	*
	July 17	2,650	742.0	28	11.45
	July 24	2,980	596.0	20	*
	July 31	2,975	476.0	16	*
	August 7	2,525	404.0	16	*

TABLE 104

Case 66. Female, manic-depressive (with diarrhea)

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	cc.		mg.
No treatment.....	August 13	1,030	412.0	40	19.75

hour collections. The recorded urinary volume then represents the true urinary excretion; the variations being caused by the great variations in fluid intake and the abnormal elimination of water through other channels.

TABLE 105

Case 84. Female, age 41 years, general paresis. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	*cc.*		*mg.*
No treatment....	August 1	1,140	273.6	24	*
	August 8	515	20.6	4	*

TABLE 106

Case 85. Female, age 38 years, general paresis. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	*cc.*		*mg.*
No treatment....	July 30	2,865	573.0	20	*
	August 6	2,455	392.8	16	*
	August 15	1,910	229.2	12	*
	August 20	2,385	381.6	16	*
	December 5	2,350	319.6	13	*

TABLE 107

Case 86. Female, age 36 years, general paresis. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	*cc.*		*mg.*
No treatment....	July 20	1,380	376.0	20	7.70
	August 8	405	97.2	24	1.88

TABLE 108

Case 87. Female, age 40 years, general paresis. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	*cc.*		*mg.*
No treatment.....	May 9	875	43.7	5	9.57

Perhaps the most striking feature of these tables is the variability of the indican excretion, a variability that has little regard for differences in any other determination. In some cases it seems generally higher on *L. acidophilus* milk than on other treatments, as in case 43, table 62; case 41, table 64; case 52, table 67, and case 51, table 80. If this were due to the tryptophan content of the milk as suggested by Smith and Kulp (164), referred to in greater detail later, the high indican excretion should accompany the ingestion of milk in all or almost all of the cases. It should also be higher at the beginning of the treatment and then should decrease as was the case in their patients and those of Kast, Short, and Croll (93). In the present series there are almost as many cases of low indican excretion with *L. acidophilus* milk as of high, and several cases in which the excretion was low at the beginning of the treatment and increased

TABLE 109

Case 88. Female, age 37 years, general paresis. Control case. Daily normal defecation

	DATE, 1923	TOTAL VOLUME	ACIDITY		INDICAN
			N/10	Per cent	
		cc.	cc.		mg.
No treatment....	July 30	625	325.5	52	2.32
	August 6	915	366.0	40	*
	1924				
	April 4,	800	176.0	22	....

TABLE 110

Summary of the total instances according to constipation, and indican excretion

MG. IN 24 HOURS URINE	CONSTIPATION	NO CONSTIPATION	GENERALLY CONSTIPATED EXCEPT DURING 3 DAYS PRECEDING ANALYSIS	NOT GENERALLY CONSTIPATED, BUT CONSTIPATED DURING 3 DAYS PRECEDING ANALYSIS
0–5	114	198	11	4
5–15	50	79	17	6
15–20	10	13	1	1
20+	4	7*	0	2

* One patient in this group had diarrhea.

after a period of weeks. Thus case 29, table 72, was on *L. acidophilus* milk for four months and on only one occasion did she excrete even a measurable amount of indican. All her other analyses showed scarcely a trace of indican in the urine. Case 38, table 75, case 48, table 82, are other examples of low indican excretion on *L. acidophilus* milk. Case 46, table 70, and case 31, table 71, show an increased indican excretion after having taken *L. acidophilus* milk for several weeks. This is contrary to the experience of previous investigators.

Constipation, judged by infrequent bowel movements, does not seem to be a factor. The number of instances of high indican excretion is practically equally divided among constipated and non-constipated cases. This is clearly shown in table 110 where the indican excretion and bowel condition are tabulated. Each analysis counts as an instance. The striking feature of this table is the infrequency of abnormal findings. Throughout the literature 5 to 20 mg. have been accepted as the normal limits. With these as standards we find only thirteen instances of abnormal excretion in over five hundred analyses; and of these—four occurred in patients who were extremely constipated, two in patients who were not generally constipated but suffered a constipated condition for the 3 days preceding the analysis, six in patients having normal daily defecations, one in a patient with diarrhea. Six of these patients then could be called constipated, and six would be non-constipated. Surely the law of chance would

TABLE 111

Summary of patients' conditions under specific treatment

TREATMENT	INDICAN, CONSTIPATION	INDICAN, NO CONSTIPATION	NO INDICAN, CONSTIPATION	NO INDICAN, NO CONSTIPATION
No treatment	0	7	3	15
Pasteurized *L. acidophilus* milk	1	3	2	1
L. acidophilus filtrate	1	3	6	3
L. acidophilus paste	3	0	4	1
L. acidophilus milk	8	7	5	6
L. acidophilus milk and lactose	4	5	5	8
Treatment discontinued	3	5	5	8
Total for all treatments	20	30	30	42
Per cent	16.4	24.6	24.6	34.4

scarcely occasion a more even distribution. If the normal limits are made less inclusive, allowing an excretion of 15 to 20 mg. to be regarded as too high, we find only twenty-five such instances or less than 5 per cent. Of these, ten occurred in constipated subjects, thirteen in patients who were not constipated, one in a patient who was generally constipated but had had good defecations during the three days preceding the analysis, and one in a patient who was not generally constipated but suffered a constipated condition during these three days. The remaining determinations were all under 15 mg., and over three hundred were less than 5 mg. It was interesting to note that the two most constipated patients, case 33, table 69, case 46, table 70, women who had had no normal movement in months, rarely showed even a trace of indican in the urine.

In Table 111 the results are tabulated not according to each analysis, but according to the general condition of each patient while taking any specific treatment. The same patient can therefore figure as more than one unit. Thus a patient starting with no treatment and then taking successively *L. acidophilus* filtrate, milk, and milk and lactose would be counted as four units, his general condition during each treatment counting as a single unit. All indican findings of less than 5 mg. are considered negligible and any patient having a preponderance of such readings on any treatment is recorded in the column headed: No Indican, No Constipation; or No Indican, Constipation, as the general bowel condition determines. From this table it will be seen that there were exactly as many patients who were constipated and eliminated practically no indican as there were patients who were not constipated and showed measurable amounts of indican. Also out of 50 constipated units only 20 units or 40 per cent eliminated any appreciable amounts of indican, while in the remaining 30 units or 60 per cent the excretion was negligible. These results indicate that indican cannot be correlated with constipation.

There have been frequent statements about the effect of diet on indican excretion. In this investigation a careful record was kept of the kind and amount of food consumed by each patient preceding and during the collection of urine. No dietary control was attempted and the patients ate freely of the routine hospital diet, of which the following menus might be considered typical:

March 1, 1923

Breakfast: Oatmeal, bread and butter, coffee.
Dinner: Soup, pork and beans, potatoes, bread and butter, milk or coffee, prunes.
Supper: Eggs, bread and butter, tea or milk.

March 2, 1923

Breakfast: Oatmeal, bread and butter, coffee.
Dinner: Soup, meat, potatoes, parsnips, bread and butter, apples.
Supper: Lima beans, buns and butter, coffee or milk.

It will be seen that the diet is fairly low in carbohydrate. Locke's figures for a man at moderately hard work are 3000 calories daily which should be derived from about 100 grams protein, 150 grams fat, and 350 grams carbohydrate. The patients in this investigation led a life of comparative leisure and the average of several days diets was 2534.8 calories. The average composition of the diet was 92.5 grams protein, 128.3 grams fat, 240.7 grams carbohydrate. While each of these is below the normal value, the carbohydrate figure shows the greatest discrepancy. When, however, 1000 cc. *L. acidophilus* milk and 300 grams lactose are administered daily the character of the diet changes, becoming predominantly carbohydrate. This was particularly true since it was noticed that patients taking the above ate very little else, as would be expected when one considers the bulk and calorific value of the milk and sugar ingested.

Thus the conditions of the experiment provide two types of diet; (*a*) a very high carbohydrate diet; (*b*) a diet in which the carbohydrate value is about 30 per cent below the normal figure supplied by Locke. If the indican were directly influenced by the diet, it was to be expected that a varied diet would cause a varied indican excretion. The record of the food consumed, however, failed to explain any of the apparent anomalies. The greater number of the controls whose diet was not characterized by the high carbohydrate of the milk and sugar excreted less than 10 mg. indican and many of them showed absolutely no indican at all by the method used. This condition was frequently duplicated among the patients whose diet consisted largely of *L. acidophilus* milk, lactose, etc. Case 40, table 86, was extremely constipated with a diet that was largely protein, and yet for over six months her urine was entirely free of indican, while case 48, table 82, was also extremely constipated, on a diet consisting principally of carbohydrate and her urine was equally free of any trace of indican. Sometimes a patient would eliminate negligible amounts of indican for months and then a single excretion of high value would occur.

Case 18, table 97, and case 33, table 69, are examples; a careful examination of their diets, bowel condition, and mental states gives no explanation of this phenomenon. Here, too, the diets, although fairly constant for each case, are distinctly opposite from each other.

Case 18, table 97, is a control patient on a low carbohydrate diet while case 33, table 69, had been on a high carbohydrate diet for months preceding the unusual indican finding. Both of these patients were extremely constipated.

Case 49, table 81, eliminated 23.80 mg. indican on June 3rd as against negligible amounts on all other analyses. However, although neither her diet nor her bowel condition explain this, she complained of not feeling well and vomited at 4.30 p.m. of the previous day.

One apparent correlation was brought out by the dietary record. Unusually high indican values frequently followed the consumption of creamed fish, clam chowder, or oyster stew. Thus case 32, table 63, generally shows negligible indican values, but on March 28th her elimination was 12.3 mg. and on July 9th it was 16.2 mg. On March 27th she ate creamed codfish for dinner and on July 8th clam chowder. The only other high figure is for May 18, 11.95 mg., for which there seems no explanation. Her highest indican value then coincides with the ingestion of fish food and the next to the highest value has a similar connection.

Case 41, table 64, shows a very high value of 24.2 mg. on May 7th, and on May 6th she ate oyster stew for dinner.

Case 42, table 66, shows an unusually high value 16.25 mg. on July 9th, following a dinner consisting largely of clam chowder July 8th. Three high values in the record of case 43, table 62, i.e. 13.09 mg. on March 8th, 18 mg. on May 7th and 10.92 mg. on July 3rd follow the ingestion of creamed codfish, oyster stew, and clam chowder.

Case 31, table 71, ate creamed codfish on March 28th and her excretion was negligible the next day, but on May 6th she had oyster stew with an excretion of 19 mg. on May 7th, and on August 5th she had clam chowder with an excretion of 16.9 mg. on August 6th. These are the highest and second highest values in her record.

Case 37, table 74, has the high value of 28.3 mg. on April 30th. Her dietary record shows that she ate four eggs the preceding day. Although this is the first instance here of high indican excretion following eggs in the diet, it is also the first instance of four eggs being eaten in one day and there may therefore be a connection between the two. On March 27th her indican value is 14.0 mg. and on May 7th it is 14.7 mg. She ate creamed fish on March 26th and oyster stew on May 6th. However, on May 16th she excreted 20.84 mg. indican with no apparent dietary connection.

These are the only occasions on which creamed codfish, clam chowder or oyster stew were eaten on the days of urine collection and each occasion is followed by a conspicuous increase in the indican excretion of one or more patients. It has been impossible to find anything in the literature regarding the tryptophan content of this food or its effect on indican excretion. It may be of interest to give the complete diets for these days.

March 7	March 27	May 6	July 8	August 4
		Breakfast		
Oatmeal Bread and butter Coffee or milk	Oatmeal Bread and butter Coffee or milk	Oatmeal Bread and butter Coffee or milk	Oatmeal Bread and butter Coffee or milk	Oatmeal Bread and butter Coffee or milk
		Dinner		
Soup Creamed codfish Barley Potato Bread Chocolate pudding	Soup Creamed fish Potato Macaroni Bread Chocolate pudding	Soup Oyster stew Potato Parsnip Oranges Milk	Clam chowder Bread and butter Potato Turnip Oranges Milk	Clam chowder Bread and butter Potato Cabbage Oranges Milk
		Supper		
Lima beans Bread and butter Tea	Rice Bread and butter Tea	Hominy Apricots Bread and butter Cheese Tea	Prunes Lettuce Cheese Buns and butter Tea	Rice Prunes Bread and butter Tea Milk

All patients took their *L. acidophilus* preparations between meals. The diet of each patient is not recorded here for lack of space, but while the choice of food was variable, each one had a helping of the codfish, clam chowder, and oyster stew. It seems evident, therefore, that aside from the unexplained effect of certain fish, diet plays no part in the indican excretion within the limits of this experiment. We have seen exactly the same indican situation in patients who have been on high carbohydrate diets as obtained in patients on low carbohydrate diets.

Practically all the published work with aciduric organisms leads to the conclusion that any successful implantation within the intestinal tract causes a sharp decrease in the indican excretion. In this investigation, no such unequivocal correlation was evident. Two other recent investigations have shown similar uncorrelative results. Smith and Kulp (164) report variable results in indican and phenol excretion on seven patients receiving *L. acidophilus* milk. They suggest that "the increased amount of available tryptophan in the milk more than balances the decrease in numbers of indol-producing organisms with the result that increased ethereal sulphate output may accompany a preponderatingly aciduric flora." Kast, Short, and Croll (93) concluded from a study of eight patients who were taking 1 liter of *L. acidophilus* milk and 100 grams lactose daily that "if indican excretion may be taken as an index of intestinal putrefaction, it appears that implantation of *L. acidophilus* in the intestine does not necessarily lower putrefactive processes in the intestine."

In table 112 indican, constipation, and bacterial records are tabulated. Although, as mentioned, both plate and microscopic counts were made, the plate counts only are recorded here as being a true measure of the number of viable organisms. The viability of the organisms on a smear preparation is open to question, but there can be no such doubt regarding plate inoculation from the feces. No case is recorded in this table in which the implantation of *L. acidophilus* is less than 20 per cent, since it was felt that putrefactive processes would not be affected by so low a percentage. It frequently happened that at the beginning of treatment a high percentage of *L. acidophilus* would be recovered from the feces before the constipation had been cleared up by the aciduric flora. This condition is noted under Remarks. It will be seen

TABLE 112

Relation between constipation, indican excretion, and the per cent of viable L. acidophilus in the intestinal canal

CASE NUMBER	DATE	INDICAN	CONSTIPATION		PLATE—PER CENT L. ACIDOPHILUS	REMARKS
			+	−		
		mg.				
51	April 18	27.30	..	−	98	....
	May 10	5.20	..	−	95	....
	May 29	*	..	−	50	....
	June 26	4.98	..	Mild diarr.	95	....
52	March 27	7.25	+	..	99	Beginning of treatment
	April 18	23.60	..	−	95	
	April 24	10.49	..	−	80	....
	May 2	14.12	Mild const.	..	90	....
	May 29	*	..	Mild diarr.	99	....
	June 26	*	..	Mild diarr.	50	....
50	April 18	15.40	Mild const.	..	100	Beginning of treatment
	April 24	14.88	..	−	50	
	May 2	12.90	..	−	30	....
	June 26	5.37	+	..	50	....
	August 13	10.90	..	−	20	....
53	March 27	14.0	+	..	50	Obstinate constipation
	April 18	29.6	+	..	40	
	April 24	*	..	−	40	....
	May 2	9.70	..	−	25	....
	May 9	8.26	..	−	90	....
	May 22	13.68	..	−	99	....
54	March 29	27.20	+	..	90	Beginning of treatment
	April 18	16.50	..	−	99	
	May 2	13.73	..	−	20	....
	May 9	13.10	..	−	60	....
	May 22	18.65	Mild const.	..	50	....
	May 29	7.86	..	−	60	....
41	March 21	7.78	+	..	100	Beginning of treatment
	April 12	27.70	..	−	100	
	April 23	13.15	..	Mild diarr.	20	....
	April 30	31.50	..	−	95	....

TABLE 112—*Continued*

CASE NUMBER	DATE	INDICAN	CONSTIPATION		PLATE—PER CENT L. ACIDOPHILUS	REMARKS
			+	−		
		mg.				
41	May 7	24.20	..	−	85	....
	June 6	6.84	..	−	50	....
37	May 7	14.70	+	..	25	Very obstinate chronic constipation of long duration
	May 9	11.58	Improving	..	35	
	May 16	20.84	+	..	50	
	May 21	*	Improving	..	10	
42	March 12	11.10	+	..	95	Beginning of treatment
	March 21	*	+	..	95	
	April 12	*	..	−	95	....
	April 30	4.43	..	−	85	....
	May 16	5.02	..	−	90	....
	May 21	20.80	..	−	75	....
31	June 6	8.54	..	−	30	....
	August 15	8.57	..	−	40	....
43	March 8	13.09	+	..	95	Very obstinate chronic constipation of long duration
	March 16	5.50	+	..	90	
	March 20	16.70	+	..	60	
	June 3	10.92	Improving	..	20	
46	May 14	*	+	..	70	Very obstinate constipation
49	June 3	23.80	..	−	65	....
	July 18	*	..	−	20	....
	August 8	*	..	−	20	....
32	May 16	5.55	..	−	95	....
	July 9	16.20	..	−	40	....
	July 17	*	..	−	75	....
44	June 6	2.54	..	−	40	....

TABLE 112—*Concluded*

CASE NUMBER	DATE	INDICAN	CONSTIPATION		PLATE—PER CENT L. ACIDOPHILUS	REMARKS
			+	−		
		mg.				
47	May 24	5.59	..	−	20	....
	August 8	4.80	..	−	50	....
33	July 11	4.30	+	..	80	Very obstinate constipation
38	February 15	2.82	..	−	100	....

+ = constipation.
− = no constipation.
* = negative or slight trace.
Mild const. = mild constipation.
Mild diarr. = mild diarrhea.

that on any extended treatment the constipation invariably improves and is generally entirely corrected.

An examination of this table reveals a striking lack of parallelism between the type of intestinal flora and the indican excretion. Case 51, table 80, shows a fecal count of 98 per cent *L. acidophilus* coincident with an indican excretion of 27.30 mg. on April 18th. This is the second day of treatment with *L. acidophilus* milk, however, and it has been found that the indican values may have a tendency to increase at the beginning of treatment. The remaining indican values are low, although the lowest indican value, June 29, coincides with the lowest number of *L. acidophilus* in the feces.

Case 52, table 67, is a conspicuous example. At the beginning of treatment his indican excretion is low, 7.25 mg. and the fecal count is 99 per cent *L. acidophilus.* During this time he is constipated. Three weeks later, April 18th his constipated condition has cleared up; his fecal count is very high, i.e., 95 per cent *L. acidophilus,* and his indican excretion is 23.60 mg. There can be no question here of high value caused by new treatment since when the treatment was begun the initial excretion was low. His indican excretion continues high—as does the bacterial count of *L. acidophilus.* His bowel condition becomes slightly constipated with no change in the indican values. This then yields to a slight diarrhea and the indican practically disappears from the urine. The last record, June 26th, showed a bacterial count of only 50 per cent *L. acidophilus* and not a trace of indican in the urine. Here the disappearance of indican may seem connected with the diarrhea, but there are numerous instances where diarrhea has been accompanied by a marked increase in indican excretion.

Case 50, table 77, shows a fairly high indican excretion, the highest value for the period coinciding with a *L. acidophilus* implantation of 100 per cent on April 18th. This was at the beginning of the treatment which may be an explanatory factor here. However, another high excretion was obtained over a month later.

Case 53, table 78, eliminates 29.60 mg. indican, his highest value April 18th when the *L. acidophilus* count is 40 per cent. One month later, May 22nd, when the *L. acidophilus* count is 99 per cent he eliminates 13.68 mg. which is also high for him.

Case 54, table 79, shows high indican values coincident with high counts of *L. acidophilus.*

Case 41, table 64, eliminates 7.78 mg. indican March 21st when her fecal count shows a transplantation of 100 per cent *L. acidophilus.* Three weeks later, April 12th, her indican excretion is almost four times as great 27.70 mg. and the *L. acidophilus* count is still 100 per cent. The indican then drops to 13.15 mg. on April 23rd and this coincides with the lowest count of *L. acidophilus,* i.e., 20 per cent. She is at this time suffering from a slight diarrheal condition. On April 30th the fecal count shows 95 per cent *L. acidophilus* and the patient's indican excretion is abnormally high, i.e., 31.50 mg. It is still abnormal on May 7th, 24.20 mg. while her intestinal flora are 85 per cent *L. acidophilus.* Then on June 6th with only 50 per cent *L. acidophilus* in her intestinal flora her indican excretion drops to 6.84 mg.

Case 42, table 66, eliminates her highest indican value for the period, i.e., 20.80 mg. on May 21st after having had a very high transplantation of *L. acidophilus* for months.

These examples suffice to show the lack of correlation between the intestinal flora and the indican excretion. Such a diversity is surprising in view of the fact that indican has been shown to be produced by the bacterial decomposition of proteins. No less surprising, however, are the number of assertions in the literature that a successful implantation of aciduric organisms in the intestine causes a conspicuous decrease in the indican excretion with little mention of bacterial counts to support the statement.

The literature of clinical psychiatry contains many statements attaching great significance to the presence of indican in the urine. True, these statements are frequently conflicting and might even be said to numerically cancel each other as far as correlating indican excretion with any particular type of mental disease is concerned. However, the general opinion is that various forms of mental disease are characterized by high indican excretion and diminished or low elimination accompanies improvement in the psychotic condition. This investigation included most of the types mentioned in the literature. Observation of the patients showed that while unusually high indican values occasionally occurred with changing mental conditions this was due to coincidence rather than correlation. The change was as often toward a state of over-activity as toward an inactive condition, but for the most part the condition remained unchanged while the indican excretion showed variation.

Case 52, table 67, dementia praecox, was slightly more manic in behavior during the period of his greater indican elimination.

Case 70, table 88, dementia praecox, shows improvement as indican excretion diminishes. Case 37, table 74, manic-depressive, on the contrary showed improvement in her mental condition when her indican excretion is highest, i.e., April 25th on. Case 31, table 71, manic-depressive, becomes sad, fearful, with ideas of death, April 25th, just at the time when her indican excretion increases. However, the indican becomes negligible in quantity with no change in her mental condition; then it increases again, although her mental condition has improved and she is much more cheerful.

Case 43, table 62, dementia praecox, also showed high indican excretion during a period of depression, stupor, and untidiness. The indican then becomes negligible despite the fact that a condition remains unchanged.

Case 49, table 81, psychoneurosis, is depressed during the whole month of May with scarcely a trace of indican in the urine. Her condition starts to improve May 27th and continues improving, but on June 3rd she eliminates 23.80 mg. of indican while her condition remains unchanged. Thus of the forty-eight patients only six gave evidence of any mental change synchronous with a variation in indican excretion, and there was absolutely no agreement between the type or the phase of the psychosis and the indican variation.

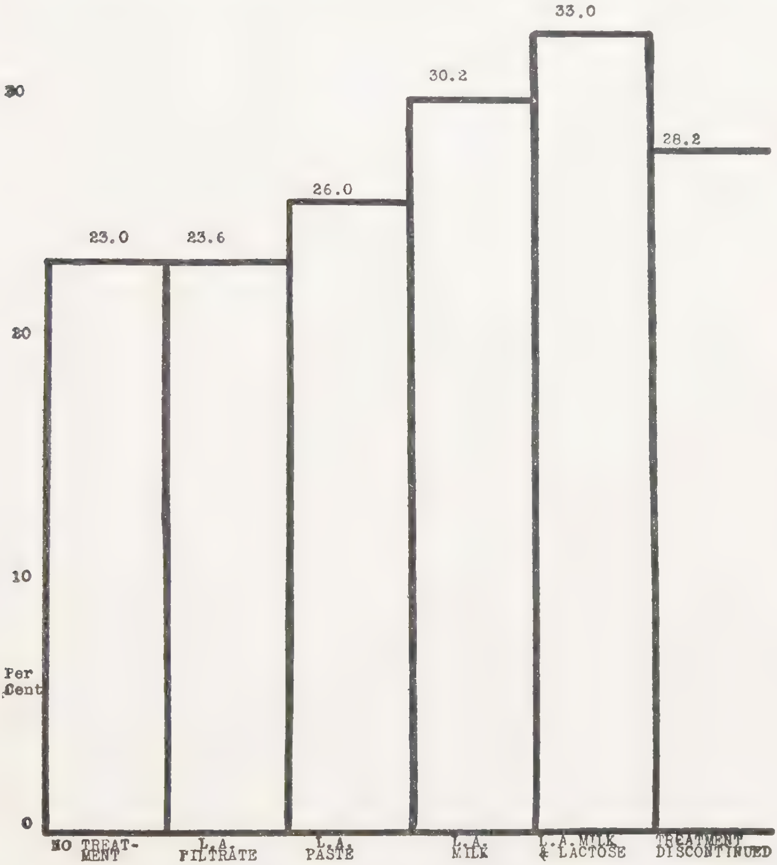

Fig. 1. Average Acidity for each Treatment

During the course of this investigation an interesting relationship was observed between the type of treatment and the urinary acidity. Though not strictly germane to the problem at hand it yet seems of sufficient importance to merit discussion. The total acidity was determined by titrating with N/10 NaOH according to Folin. It was found that the total acidity varied with the lactic-acid-producing ability of the preparation ingested. This is graphically represented in figure 1. On the abscissa are recorded the various treatments. Pasteurized *L. acidophilus* milk is omitted because the acidity of this preparation was neutralized before pasteurization in order to prevent extreme coagulation of the casein. After the present experiment was in progress the preparation was brought up to its original acidity by the addition of lactic acid after pasteurization, but none of the patients taking this product are here included. On the ordinate is recorded the percentage acidity. The record under each treatment represents the average of the percentage acidity of all patients on that treatment. As previously explained, *L. acidophilus* filtrate is prepared by passing regular *L. acidophilus* milk through a Mandler diatomaceous filter. This leaves the filtrate sterile but chemically of about the same acidity as the original *L. acidophilus* milk. This was generally about tenth normal, i.e., 10 cc. milk was neutralized by 20 cc. of N/20 NaOH. The solid residue in the Mandler filter contains all the viable organisms in a close packed mass. This is not very acid but is capable of producing lactic acid in the intestine because of the active *L. acidophilus*. The organisms scarcely function, however, since the greater part of the nourishing ingredients of the pabulum has been withdrawn in the filtrate. *L. acidophilus* milk contains an average of 200,000,000 organisms per cubic centimeter actively functioning in a favorable medium. Its acidity on ingestion is only N/10 but it has the ability to produce lactic acid in the intestine. *L. acidophilus* and lactose provide the optimum condition for the production of lactic acid. It will be seen that on "no treatment" the average acidity is 23.6 per cent. On the sterile *L. acidophilus* filtrate the acidity remains unaltered. When the biologically active *L. acidophilus* paste is fed, the urinary acidity is increased to 26 per cent. The ingestion of *L. acidophilus* milk causes a sharp rise in acidity to 30.2 per cent and this is still further increased on the addition of lactose to 33 per cent. When the treatment is discontinued the acidity drops to 28.2 per cent. This is higher than the average acidity under "no treatment" caused by the fact that many of the figures making up the average of "treatment discontinued" were obtained immediately after the milk and lactose regimen and it was noticed that the acidity remained high for some time. Since this treatment generally caused an implantation of aciduric flora in the intestine the persistence of highly acid urines and the subsequently high average acidity are easily understood. Of course, the graphic representation, being constructed from the averages of all determinations for each treatment gives no idea of the range of individual response. The correlation between the "potential acidity" of the preparation ingested and the acidity of the urine is clearly demonstrable whenever different types of treatment have succeeded each other. To show this correlation the records of patients who have taken three or more preparations have been tabulated in table 113. The average acidity for each patient on

TABLE 113

Acidity averages of patients on three or more treatments

CASE NUMBER	NO TREATMENT	L. ACIDOPHILUS FILTRATE	L. ACIDOPHILUS PASTE	L. ACIDOPHILUS MILK	L. ACIDOPHILUS MILK AND LACTOSE	TREATMENT DISCONTINUED
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
41	...	..	Sl. acid*	38	..	25
52	24†	..	..	35	52	38
58	...	31	..	60	..	49
33	16	..	6	37	34	..
46	...	18	..	21	30	..
31	Sl. acid†	..	..	22	32	..
29	...	Sl. acid*	..	17	..	14
50	8†	14	..	32	40	23
53	Neutral†	..	24	27	64	44
54	24†	..	47	50	53	45
51	12†	8	..	19	32	15
49	...	17	..	24	29	..
48	...	38	..	32	11	..
44	...	38	28	40	..	45
47	...	..	10	21	22	7

* Slightly acid: determination made before quantitative titration was instituted.

† This average is for "Pasteurized milk," but since the acidity of the preparation was previously neutralized, its acidity effect is comparable to that of "No treatment."

any treatment is recorded under that treatment. Averages are calculated to the nearer whole number. The first column, headed "Table number" gives the number of the table containing the complete record for that patient.

It will be seen that in nearly every case there is a sharp increase in urinary acidity with the ingestion of *L. acidophilus* milk which is augmented on the addition of lactose. The effect of *L. acidophilus* paste is not a consistent one, but this was scarcely to be expected with so unfavorable a medium for bacterial activity. That the acidity depends largely upon the lactic-acid-producing organisms, or what has been called the "potential acidity" of the preparation is shown by the persistence of highly acid urines after treatment has been discontinued. Reference to table 112 shows a high count of *L. acidophilus* in almost all cases of a persistently high acidity. Thus case 41, table 64, shows a very high per cent of *L. acidophilus* in the fecal count, as do case 52, table 67, case 33, table 69, case 46, table 70, case 53, table 78, case 52, table 79, case 51, table 80, and for the most part case 50, table 77, case 31, table 71, case 49, table 81, case 44, table 83, case 47, table 84, have fairly good implantations of *L. acidophilus* as evidenced by their fecal counts. It will be seen that the urinary acidity of these patients increases with the acidity of the preparation ingested. Cases 58, 29, and 48 are not represented in table 112. The attempt to change their intestinal flora to the aciduric type was not very successful. Case 58, table 68, is the only one of the three whose urinary acidity on different treatments follows the general curve with any great fidelity. The variations in the acidity of case 29, table 72, are so slight as to be negligible, while the acidity of case 48, table 82, is completely anomalous.

The interesting work of Taistra (166) and more especially of Blatherwick and Long (22) show definitely that the acidity of the urine is directly affected by the ingestion of large quantities of lactic acid. Taistra fed glucose, acetic acid, lactic acid, glycollic acid, and hydrochloric acid in broth to a dog. It was found that while the glucose and acetic acid had no effect on the acidity of the urine, the ingestion of glycollic acid and of lactic acid resulted in a conspicuously increased acidity. Also the carbon dioxide-combining power of the blood remained unaffected by the glucose and the acetic acid; whereas the lactic acid, glycollic acid, and hydrochloric acid produced a profound depression in the CO_2-combining power. This is caused by a gain in acid radicals which is compensated for by an augmented elimination of acid through the kidney; hence the increased acidity of the urine. Blatherwick and Long worked with human subjects. They found that "the ingestion of such large amounts (500 cc.) of lactic acid milk is capable of reducing the alkaline reserve by the entrance of the acid into the blood." This results in an increased acidity of the urine in the form of acid phosphate. These results indicate the need for caution in prescribing large quantities of lactic acid milk, particularly if there already exists a tendency towards acidosis.

SUMMARY AND CONCLUSIONS

Over five hundred urinalyses were made on forty-eight psychotic patients. Twenty-three of these patients were receiving various *L. acidophilus* preparations for the alleviation of constipation; twenty-five were control cases receiving no treatment. Of the controls four were extremely constipated, one had diarrhea, and the remaining twenty enjoyed good daily defecations. The following determinations were made on twenty-four hour specimens of urine: volume, specific gravity, color, acidity, and indican according to the method of Meyers and Fine. A record was kept of the kind and amount of food consumed by each patient daily, and the number and quality of bowel movements. The fecal flora were determined weekly, both plate and microscopic counts. The experiment was carried on for a period of about one year.

Within the limits of this investigation the following conclusions seem justified.

1. There is no correlation between constipation, as evidenced by infrequency of bowel movements, and indican excretion in the urine. It is generally agreed upon in the literature that the normal indican excretion is between 5 and 20 mg. daily. This has been accepted in order to keep the results as easily comparable to previous work as possible. Only thirteen instances of abnormal excretion were found in over five hundred analyses. Of these six occurred in constipated subjects, six in subjects having normal daily defecations and one in a patient with diarrhea. There were only twenty-five instances of an excretion of 15 to 20 mg.;

ten occurring in constipated subjects, thirteen in patients having normal daily defecations, and two in patients whose classification was questionable. The remaining determinations were all under 15 mg., about three hundred being less than 5 mg. Obviously "normal" can be used here only as opposed to "pathological" since in the sense of "common" or "ordinary," the normal limits should be placed at 0 to 10 mg.

2. The indican excretion is no more affected by a high carbohydrate diet than by one comparatively low in carbohydrate. Patients whose diet consists mainly of 1000 cc. *L. acidophilus* milk and 300 grams lactose daily are unquestionably on a high carbohydrate diet, while those whose diet contains 30 per cent less than the normal carbohydrate allowance are equally unquestionably on a very much lower carbohydrate diet, one which may even be called "low" as compared with normal figures. These two diets offer contrasting conditions under which the indican situation remained practically unchanged. The literature is replete with statements concerning the telling effect of high carbohydrate diets on indican excretion, but the high carbohydrate diet of this experiment seemed to have little effect. There were not enough cases of abnormal indican excretion to determine the effect of a high carbohydrate diet under such conditions, but many of the patients showed indican values that were quite high for them while taking *L. acidophilus* milk and lactose. The inclusion of creamed codfish, clam chowder, and oyster stew in the diet was frequently followed by an unusually high excretion of indican.

3. There was no correlation between an aciduric flora in the intestine and the indican excretion.

4. There was no correlation between the mental condition of psychotic patients and their indican excretion.[3]

5. The ingestion of lactic acid preparations and lactose caused the formation of strongly acid urines. The acidity of the urine was found to vary with the lactic-acid-producing ability of the preparation.

[3] Particular thanks are due Dr. V. C. Myers and Miss Lela Booher of the New York Post-Graduate Medical School for suggestions regarding the indican method used.

CHAPTER IX

Practical Recommendations

Since the beneficial effects in constipation and diarrhea which may be obtained by the use of *L. acidophilus*, have been demonstrated experimentally, certain practical recommendations may be made to the physician and layman interested in such therapy. Striking as the results are, *L. acidophilus* is no panacea for all ills, nor is there any guarantee that it will prolong life to the extent that Metchnikoff hoped. As with any other therapeutic agent one must first have an adequate comprehension of the condition and the individual to be treated. The medical treatment of constipation is not easy in that it usually requires a considerable degree of coöperation on the part of the patient, particularly as regards restrictions on diet, exercise, etc. Many patients follow the regimen faithfully enough for a short period of time only to arrive at a point where the cure seems worse than the ailment, whereupon there is a reversion to former bad habits. It is not to be presumed that *L. acidophilus* therapy is more efficient in alleviating constipation than the usual medical treatment, rather, it furnishes a short-cut which is usually more acceptable to the patient. Most individuals have a liking for buttermilk, and the palatability of *L. acidophilus* is similar. Even in individuals who have no liking for sour milk, a taste for it may be cultivated by adding some pleasant flavor, such as vanilla, chocolate, pineapple, lemon, etc. Finally, *L. acidophilus* may be administered rectally where oral administration must be supplemented, or is undesirable.

Obviously, the type of *L. acidophilus* treatment varies with different individuals and is dependent upon the condition to be treated. In general, the average daily dose of *L. acidophilus* milk should be the equivalent of 1000 cc. of a culture containing approximately 200,000,000 viable *L. acidophilus* per cubic centimeter. In milder cases of constipation this dose may soon be reduced, in more severe cases it may be increased. Since when ingested for the first time, *L. acidophilus* milk causes the formation of considerable gas, it has been our practice to start with 500 cc. the first day; 750 cc. the second day and 1000 cc. the third day. Fifty grams of lactose is added on the third day and the dose is increased 50 grams each day up to 300 grams (about $\frac{3}{4}$ pound) per day. Ordinarily this dose of 1000 cc. of *L. acidophilus* milk and 300 grams of lactose will produce a good response within two weeks. As stated before, the milk may be flavored to suit the taste without influencing its value. As the patient's condition is improved, the dose may be gradually diminished. Frequently we have found that 100 grams of lactose (about $\frac{1}{4}$ pound) per day with 1000 cc. of *L. acidophilus* milk once a week or even less frequently, is sufficient to maintain the benefits of the more intensive course of treatment. Where the patients do not respond readily to treatment, the dosage of milk may be increased to 1500 cc. per day and the lactose to 400 grams (about 1 pound). In addition an enema of 1000 cc. of *L. acidophilus* milk thinned slightly with warm water to permit easy flow, may be given following a preparatory high cleansing enema. Such a procedure is of value in diarrheal cases. Lactose, is of course contraindicated in diarrhea. During *L. acidophilus* treatment it is advisable for the patient to abstain from the use of cathartics and purgatives of all kinds. Enemas and glycerine suppositories may be employed to furnish relief when required.

While *L. acidophilus* milk has proven efficacious in the treatment of constipation, diarrhea, sprue, tropical bacillary dysentery, etc., its value in obstipation and amebic dysentery is doubtful. Patients suffering from gastric hyperacidity are rarely good subjects for *L. acidophilus* therapy. The use of *L. acidophilus* in children's intestinal complaints should prove satisfactory. There is indeed every reason to believe that it would be particularly advantageous in the summer diarrheas which are so difficult to combat. As a rule children tolerate fermented milks, so that there would be no objection to feeding them a reliable pure culture of *L. acidophilus*. Such an experiment is being undertaken.

The proposed use of *L. acidophilus* milk invariably raises questions concerning diet. It may be said that one of the most agreeable features of *L. acidophilus* treatment is that a change in diet is not essential. Naturally, it is desirable that animal proteins be reduced to a minimum, yet total abstention is not necessary. The addition of so much milk and lactose to the usual daily diet will ordinarily interfere with the appetite. However, since the nutritive value of milk and the calorific value of lactose is high, there need be no fear on the score of insufficient nourishment during *L. acidophilus* feeding. It is advisable to administer divided doses of *L. acidophilus* as nearly midway between meals as possible, in order not to affect the appetite any more than necessary. In general, patients gain weight on *L. acidophilus* milk and lactose. When this is undesirable, it is best to reduce the dosage of lactose.

Any one who recommends *L. acidophilus* treatment is placed in an anomalous position because of the nature of the commercial preparations now on the market. Bluntly stated, they are almost all worthless. Obviously *L. acidophilus* tablets now on the market are of no value when compared with viable milk cultures. As Bass (12) has stated:

> In the instance of tablets, none of those examined was found to contain as many as 1000 viable bacteria of any kind per tablet. If it should be granted that all the viable bacteria present were *B. acidophilus*, it would take about 1,000,000,000 tablets or more than 20 tons, to contain as many bacilli as are contained in 1000 cc. or the usual daily dose of the *acidophilus* milk, the quantity found, by most investigators at least, to be necessary to transform the flora.

Our experience, as well as that of other investigators, corroborates these findings.

Liquid cultures of various description are tastefully labelled; they may contain broth, pineapple, or other juices, etc., yet none of them can stand comparison with a laboratory-made culture of *L. acidophilus*. To quote Bass again (12): "Bacteria were numerous in the commercial liquid cultures examined. If they were all *B. acidophilus*, a patient would have to drink only about 7 to 8 gallons daily to get as many as he would in 1000 cc. of the *acidophilus* milk culture." From time to time ingenious preparations purporting to contain viable *L. acidophilus* in large numbers appear on the market. Frequently the menstruum is of undoubted value as in the case of mineral oil suspensions; again it may be worthless as in the case of chocolate candies. Viable *L. acidophilus* organisms, however, are conspicuous by their absence.

There remain a limited number of *L. acidophilus* milk preparations which do contain viable *L. acidophilus* organisms but in numbers relatively far below laboratory cultures. It has been pointed out that the nature of *L. acidophilus* therapy is chiefly bacteriologic (or biochemical). This means that the larger the inoculation the greater will be the effect (within certain limits). Essentially, *L. acidophilus* therapy is a mass-inoculation phenomenon and a reduction in numbers of viable organisms is accompanied by a corresponding diminution of activity. This cannot be overemphasized, for the clinical effects are immediately noticeable. Curiously, other investigators have, as a rule, failed to indicate the number of viable organisms they administer. This is a matter of importance, both practically and scientifically and one which may presumably account for certain discordant results.

Finally, capital has been made of the inferior quality of commercial *L. acidophilus* preparations to further the sale of various sugars. The indubitable value of lactose as an aid in *L. acidophilus* therapy is recognized. But lactose alone cannot replace *L. acidophilus* milk. Further, any combination of lactose with other sugars cannot be superior to the additive value of the same sugars taken separately. Undoubtedly there are methods for improving the palatability of such sugars as lactose, dextrin, etc., but there may be considerable question as to whether the premium paid is worth while, compared with the cost of sugars of U. S. P. quality as marketed by reliable chemical houses. The "c.p." grade of sugars is not required for the purposes under discussion.

To sum up: Constipation, diarrhea and other intestinal disorders may be satisfactorily treated by *L. acidophilus*. Such treatment is simple and works no hardship on the patient. The use of *L. acidophilus* should for the present be limited to preparations from scientific laboratories rather than extended to the unsatisfactory products now being exploited commercially.

CHAPTER X

General Summary

The claims made by Metchnikoff and his colleagues concerning the therapeutic value of *L. bulgaricus* are based on little experimental data that can bear careful scrutiny. Many critics, however, furnish evidence which is no more convincing that that supplied by the adherents of Metchnikoff's theories. On the other hand the literature on *L. acidophilus* is sufficiently sound to show that this organism, unlike *L. bulgaricus*, can be easily recovered from the gastro-intestinal tract. The bulk of reliable evidence goes to prove that a simplification or transformation of the intestinal flora from a proteolytic to an aciduric type, dominated chiefly by *L. acidophilus*, may be induced by the feeding of suitable carbohydrates or the ingestion of viable *L. acidophilus* organisms. Direct clinical observations, as well as indirect laboratory methods, have indicated the practical importance of *L. acidophilus* and its therapeutic possibilities. It remained for the investigators from Yale University to adduce the most important body of information concerning *L. acidophilus* and *L. bulgaricus*. They were successful in implanting *L. acidophilus* in the intestine of animals as well as man and demonstrated its therapeutic application in certain intestinal disorders.

Experimentally, we have shown that *L. bulgaricus* can rarely, if ever, be implanted in the human intestine. In only one instance out of twelve were *L. bulgaricus* organisms recovered in the feces after two weeks of feeding in large quantity. *L. acidophilus*, however, was recovered from all of the same patients, ingesting this organism in large numbers. Constipation was markedly alleviated by such administration.

The benefits of *L. acidophilus* milk were further indicated in a series of cases suffering from constipation and diarrhea and the transformation from proteolytic to aciduric flora noted. It was found that *L. acidophilus* milk may be kept satisfactorily for only about 3 to 4 days. In extending the series of patients under

L. acidophilus treatment to fifty-six, the same striking increase in number of normal defecations was observed in a majority of instances. Patients who had previously averaged not more than one normal defecation per week had normal defecations almost daily. Lactose proved to be valuable in prolonging the beneficial effects of *L. acidophilus* milk feeding after the administration of viable *L. acidophilus* organisms was discontinued. Eighty per cent of thirty-four patients had a larger number of normal defecations long after treatment was discontinued than before it was begun. Eleven cases of severe diarrhea, not of infectious type, and two cases of sporadic diarrhea were successfully treated by oral administration of *L. acidophilus* milk, augmented when necessary, by rectal administration. Complete defecation records have been recorded for each patient under observation throughout all these investigations in contradistinction to mere expression of opinion as to results obtained.

The inquiry into the nature of *L. acidophilus* therapy showed that its *modus operandi* was not a physical phenomenon, since patients receiving sterile milk were not relieved of constipation. Neither does it appear to be a strictly chemical phenomenon since patients receiving pasteurized *L. acidophilus* milk were not relieved of constipation. In order to check this up even more closely, *L. acidophilus* milk was centrifuged and run through a Mandler diatoneaceous filter, a process in which the chemical composition should not suffer alteration. When fed to constipated patients this filtrate was practically without effect. Regular *L. acidophilus* milk ingested subsequently resulted in an increase in the number of normal defecations. When sterilized *L. acidophilus* milk with an addition of lactic acid to approximate the original chemical composition was fed to constipated patients, little change was noted. While again, a subsequent ingestion of *L. acidophilus* milk resulted in an increase in the number of normal defecations. These data indicate that *L. acidophilus* therapy is essentially bacteriologic rather than physical or chemical in nature.

In an investigation of the significance of indican in a series of 48 cases there was found to be no correlation between constipation, as evidenced by frequency of defecation and indican excretion in the urine. Only 13 instances of abnormal excretion of indican (normal: 5 to 20 mg.) were found in over 500 urine analyses.

Of these six were constipated subjects, six in subjects having normal daily defecations and one in a patient with diarrhea. Indican was not affected by changes in carbohydrate diet, i.e., lactose, etc. There was no correlation between an aciduric flora in the intestine and indican excretion. The ingestion of lactic acid preparations and lactose did cause the formation of strongly acid urines in proportion to the lactic-acid-producing ability of the preparation.

From a practical standpoint it has been shown that constipation, diarrhea, and other intestinal disorders may be satisfactorily treated by *L. acidophilus*. Such treatment is simple and works no hardship on the patient. The use of *L. acidophilus* should, for the present, be limited to preparations from scientific laboratories rather than extended to the unsatisfactory products now being exploited commercially.

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APPENDIX

PART I

PREPARATION OF L. ACIDOPHILUS MILK[1]

Sterilise fresh skimmed milk or fresh whole milk from which the cream has been removed, at 20 pounds pressure for 20 minutes until the milk has become slightly caramelized. Our practice is to make transfers of the original strain into about one-half dozen 500 cc. Erlenmeyer flasks containing about 250 cc. of milk. These are known as resting cultures. Examine the original cultures received for purity. There should be a Gram-positive fairly thick rod, variable in size, usually rather long, occurring in long chains. Thanks to the courtesy of Dr. Morton Charles Kahn our cultures now represent the pedigreed progeny of a single cell which we fish by the Barber-Chambers technic described by Chambers and Kahn in the Jour. Inf. Dis., 31, 1922, 334–348.

Incubate the resting cultures at 35° to 37°C. until the milk has coagulated and forms a soft curd which comes away characteristically as a homogeneous mass from the side of the flask when tilted (see plate I). As a rule there will be found a layer of whey covering this curd in about 24 hours. The fermented milk has a characteristic, bland, slightly acid odor and taste. If putrifying odor develops the milk should be discarded. Now transfer, after thoroughly shaking, approximately 2 cc. of coagulated milk into 100 cc. of sterilized milk and incubate at 35°C. from 36 to 48 hours. When properly coagulated it is ready for use as an inoculum but its purity should be first established by microscopic examination. It is our practice to daily inoculate freshly sterilized milk with the previous day's coagulated milk for about two weeks. When the organism fails it is advisable to use another resting culture. As mentioned before we keep at least one-half dozen resting cultures on hand at *room temperature*, and never keep a resting culture more than a month. Use new fecal strain every two to four months. One cannot be too careful about the aseptic transfer of this lactobacillus. A Bunsen flame is inverted into the mouth of the flask when transferring, etc. Our modified Gram stain (see part III) will be found particularly advantageous for identifying purity of the fermented milk. Whey agar plates (or ordinary nutrient agar) are used for very low dilution (1:10) of milk to establish purity and high dilution on whey agar to count the number of viable organisms per cubic centimeter. This is prepared according to Rettger and Cheplin (144) as follows:

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[1] Reprinted from the State Hospital Quarterly (N. Y.) *10*, August, 1925, 584–589.

PREPARATION OF WHEY AGAR

"The skimmed milk is heated to 80 to 90°C., and 5 cc. removed to a test tube; while the test sample is still hot it is treated with 10 per cent solution of hydrochloric acid drop by drop until complete coagulation takes place. This is repeated two or three times. The calculated amount of acid required to coagulate all of the milk is thoroughly mixed with the milk, the temperature of which is still 80 to 90°, and the casein allowed to settle. This is followed by filtration, at first through absorbent cotton and then paper, and neutralization of the filtrate with sodium hydroxide to pH 6.8 to 7.0. Before further filtering, 0.5 per cent peptone is added[2] and the medium is autoclaved for fifteen minutes at 15 pounds extra pressure. The albuminous material is filtered off through paper, and the clear filtrate employed as such for whey broth or for the preparation of whey agar. To convert into whey agar add 1.2 per cent of standard shredded or powdered agar to the whey broth."[1]

EQUIPMENT

Autoclave; incubator; 500 cc. Erlenmeyer flasks; 1 litre Erlenmeyer flasks. Microscope; slides; immersion oil; methyl violet; alcohol; acetone; sodium bicarbonate; iodine; sodium hydroxide; basic fuchsin; xylol. Petri dishes 100 × 15 mm.; agar; hydrochloric acid, 10 per cent; sodium hydroxide, N/20, and N/1; lactose c.p., peptone, egg albumen (powdered). Absorbent and non-absorbent cotton.

DOSAGE

As for dosage of *L. acidophilus* milk, administer 300–500 cc. the first day; 500–750 cc. the second day; 1000 cc. daily thereafter. This is further reinforced by lactose (U. S. P.) in the following dose: On the third day 50 grams of lactose are added to the milk at the time of taking; 100 grams the fourth day; 200 grams the fifth day; 300 grams the sixth day and 300 grams daily thereafter unless diarrhea or undue gas is formed. Some little gas will be formed the first few days. The dosage should be reduced after regularity is established as appears desirable. Frequently milk twice per week will suffice when lactose is taken daily.

The fermented milk should be taken between meals so as not to interfere with the appetite. The 1000 cc. may be divided into two or more portions if desired. Naturally, when one is taking this extra quantity of food, the diet will voluntarily be reduced. No other modification of diet is required other than that the animal proteins should be reduced to a minimum. The palatability of the product varies with the acidity developed.

A daily record should be kept for each patient, giving the date, the amount of milk taken, the amount of lactose taken, the number of bowel movements, as well as their size, color, and consistency, so that the results may be properly evaluated.

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[2] It is our practice at this point to mix in thoroughly 0.3 per cent powdered egg albumin, which has been previously dissolved in about 20 cc. of water (by allowing to stand over night at room temperature).

In diarrhea, lactose is contraindicated. In stubborn cases of chronic constipation and diarrhea high enemas of *L. acidophilus* thinned with a little warm water may be used in conjunction with oral administration. Precede such treatment with a cleansing enema.

LARGE SCALE PRODUCTION

For large scale production, vertical pasteurizing tanks or the following procedure as suggested by Bass (14) may be used. (It is believed that a large metal steam cooker such as used in the N. Y. State Hospital kitchens could be similarly employed. It would, however, have to be fitted with a cold water coil.) The skim milk is raised to a temperature of 190° to 195°F. and held at that temperature for one hour. It is then cooled to about 98°F. and held at this temperature for 3 to 4 hours. Then the temperature is again raised to 190° to 195°F. for one hour. Again the milk is cooled to 98°F. and inoculated with 2 per cent of pure *L. acidophilus* milk culture. It is held at 98°F. for 36 hours. The coagulated milk is then broken up by a sterile mechanical agitator or paddle. It is cooled to room temperature and distributed immediately. Keeping *L. acidophilus* milk in the icebox kills off many viable organisms. It is best to keep it at room temperature.

FECAL ANALYSIS

The method of making fecal analysis as employed by us is as follows: After having been requested to urinate, the subject is seated in a "commode" chair (equipped with a pail) over a piece of cheese cloth about one foot square, fastened by means of adhesive tape. The gauze containing the specimen is then transferred to a 2 quart "oyster leader" or pasteboard carton, noting thereon, name, date, hour of collection and whether obtained normally or by suppository. When specimens cannot be obtained normally, soft pure glycerine suppositories are employed sometimes using two or three, allowing ten minutes to elapse. Fecal specimens are brought to the laboratory as soon after collection as possible. Delay beyond an hour or two should not be countenanced.

Ten grams of the specimen are weighed into 90 cc. of sterile physiological saline solution in an Erlenmeyer flask. This is shaken for 10 to 15 minutes in an electrical shaking machine. Of this 1:10 dilution, 1 cc. is transferred by a sterile pipette to 99 cc. of saline and shaken. Of this 1:1000 dilution, 1 cc. is again transferred to 99 cc. of saline and shaken. Of this 1:100,000 dilution, 10 cc. are transferred to 90 cc. of saline and shaken. Duplicate 1 cc. portions of this 1:1,000,000 dilution and of the 1:100,000 dilution are placed in sterile Petri dishes and 10 cc. of whey agar at 42°C. added. Incubation is from 4 to 7 days at 37°C.

For the microscopic examination, a small amount of feces is rubbed with a sterile glass rod in a test tube containing from 5 to 10 cc. of physiological saline solution until the turbidity approximates tube 12 of McFarland's nephelometer scale (as described in the Journal of the American Medical Association, *49*, 1907, 1176-1178) prepared by adding 12 cc. of a 1 per cent barium chloride solution to 88 cc. of a one per cent sulfuric acid solution. Using a "hydrogen ion block" for comparisons a tube of 0.15 per cent aqueous solution of potassium dichromate is employed to compensate for the color of the feces. A drop of the fecal suspension is placed on a microscopic slide and Gram-stained according to the method described in part III of this appendix.

PART II

SOME TEMPERATURE STUDIES ON L. ACIDOPHILUS MILK[3]

Since the beneficial effects of *L. acidophilus* appear to depend upon a transformation of the intestinal flora it follows that a mass inoculation is desirable. For this purpose sterilized skim milk inoculated with *L. acidophilus* seems to be the most satisfactory medium, although some investigators have other preferences. In any event, the keeping quality of the pabulum is of prime importance. The principal factors which must be considered in this connection are palatability, and the number of viable *L. acidophilus*. Palatability in the case of fermented milk depends upon the development of acidity and is largely a matter of individual taste. In general it may be said that for those who do not find fermented milk unpalatable an acidity titratable by 25 cc. of N/20 NaOH per 10 cc. of milk represents about the upper limit. The number of viable organisms present in any medium is a function of the time and temperature of incubation, as well as of acidity. In a previous communication (105) data have been presented dealing with the keeping quality of *L. acidophilus* milk at room temperature. However, the recommendation usually appearing on commercial preparations of fermented milk is that such milk be kept in a cool place, preferably in the ice-box. Consequently, it was considered advisable to investigate the influence of low temperature on the number of viable *L. acidophilus* in milk. In this study therefore, the following procedure was employed. Three Erlenmeyer flasks (500 cc. capacity), containing 300 cc. of skim milk were sterilized in the autoclave in the usual manner at 20 pounds pressure for 20 minutes. To each of these flasks on cooling were added 10 cc. of milk containing approximately 200,000,000 viable *L. acidophilus* per cubic centimeter. The flasks were then incubated at 35°C. for 24 hours. The contents of each flask were plated in triplicate in a dilution of 1:1,000,000 on whey agar. (All plates were incubated at 35°C. and read at the end of 3 to 4 days.) The flasks were then placed in the ice-box which maintained a temperature of about

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[3] Reprinted from State Hospital Quarterly (N. Y.), 8, May, 1923, 372-381.

9°C. (8° to 10°C.). The contents of each flask were plated in triplicate in the usual way every 24 hours. Thus the number of viable *L. acidophilus* was determined and the per cent killed (of the number originally present) was calculated. This experiment was repeated three times and the results are included in table 114. The figures under the heading of "number of viable *L. acidophilus*" represent the averages of triplicate platings from triplicate flasks. Each set of triplicate flasks is designated as Part I, II, III, in this table. The probable error of these determinations has been calculated in the usual way

TABLE 114

Acidophilus milk at ice-box temperature (9°C.)

ORIGINAL COUNT*	NUMBER OF DAYS IN ICE-BOX	NUMBER OF VIABLE L. ACIDOPHILUS*	PER CENT KILLED
I			
148	..	148	..
	1	77	48
	2	24	84
	3	5	97
II			
213	..	213	..
	1	114	46
	2	56	74
	3	18	92
	4	6	97
	5	0.6	99
III			
230	..	230	..
	1	130	43
	2	90	61
	3	53	77
	4	18	92
	5	Lost	..
	6	6	97
Average.......	1	...	46
	2	...	73
	3	...	89
	4	...	94
	5	...	99
	6	...	97

$$\text{P.E.} = \frac{\text{average deviation}}{\text{number of instances}}$$

and averages ± 9 per cent. The experimental error in such investigations must necessarily be great because of the difficulty of obtaining a uniform suspension of coagulated milk.

It will be seen in table 114 that in the first instance, where the original count was 148,000,000 *L. acidophilus* per cubic centimeter there was a striking reduction in numbers on successive days after ice-boxing. Thus after one day almost one half the number of viable organisms were killed; after 2 days, 84 per cent, and after 3 days, 97 per cent were killed. Similar results were obtained in the next two repetitions of this experiment when platings were continued for 5 and 6 days respectively as shown in parts II and III of table 114. The average per cent on successive days in the above experiments is calculated at the bottom of table 114. It is evident that after 3 days in the ice-box there are very few viable *L. acidophilus* present since about 90 per cent of the original number are decimated.

The importance of having a large number of viable organisms present is obvious. Frequently in treating severe cases of chronic constipation it is necessary to increase the dose before obtaining beneficial results. Furthermore, it is likely that in cases reported as failures a sufficient increase in the number of viable organisms administered might have resulted in success. In fact it is difficult to interpret clinical experience with *L. acidophilus* unless a careful record has been kept of the actual number of viable organisms ingested. While standardization of mass inoculation can scarcely be achieved nevertheless our experience would lead us to believe that the daily dose should ordinarily be about 200 billion viable *L. acidophilus*, which is represented by 1,000 cc. of milk containing 200 million viable *L. acidophilus* per cubic centimeter. When the total daily number of viable *L. acidophilus* administered drops considerably below 100 million per cubic centimeter the therapeutic effects become less noticeable.

It would appear therefore that the practice of ice-boxing *L. acidophilus* has little to recommend it beyond preserving the palatability of the culture. Such practice reduces the number of viable organisms to a negligible quantity which seriously interferes with the beneficial results to be expected. It is more desirable to keep *L. acidophilus* products at room temperature.

There has been some difference of opinion as to the proper degree of sterilization to which milk should be subjected before inoculation with *L. acidophilus*. Our experience confirms that of Rettger and Cheplin (144) that the milk should be sterilized until it becomes a light caramel color. Autoclaving at 15 pounds pressure for 20 minutes or more is required. The following experiment was planned to study the influence of time and pressure in autoclaving milk prior to inoculation with *L. acidophilus* with respect to the growth of the organism. One hundred and fifty cubic centimeter portions of skim milk in 300 cc. Erlemeyer flasks were subjected to 15 pounds pressure for 15, 20, 25, 30, 40, 50 and 60 minutes, and 20 pounds pressure for 10, 15, 20, 25 and 30 minutes respectively. Each flask upon cooling was inoculated with exactly 10 cc. of milk containing approximately 200 million *L. acidophilus* per cubic centimeter. The flasks were incubated for 24 hours at 35°C. and the contents then plated in triplicate on whey agar. Microscopic examinations were made of the organisms stained by the modified Gram method described in part III of this appendix. The color, taste, and acidity, of the milk were also recorded. In order to determine what influence acclimatization might exert on the number of *L. acidophilus*, 10 cc. from each flask was used as an inoculum into another flask of milk sterilized at the same period of time and at the same pressure. After 24 hours incubation at 35°C. the contents of these flasks were plated and Gram-stained. This procedure was again repeated, subcultures being made from these flasks to milk correspondingly sterilized. In this way, it was hoped that some conclusion might be reached as to the most desirable time and pressure in the autoclave for the sterilization of the milk, since three subcultures

TABLE 115

The influence of time and pressure in the autoclave on the growth of L. acidophilus in milk

NUMBER OF MINUTES	NUMBER L. ACIDOPHILUS PER CUBIC CENTIMETER*			COLOR	TASTE	N/20 NaOH PER 10 CC. MILK
	First day	Second day†	Third day†			
			15 pounds pressure			
						cc.
15	102	127	187	V. f. brown	Routine	12.8
20	137	101	195	F. brown	Routine	14.2
25	80	84	148	F. brown	Routine	13.9
30	246	133	108	F. brown	Routine	13.6
40	116	45	62	L. brown	Routine	14.7
50	4	45	35	M. brown	S. caramel	13.0
60	21	103	48	D. brown	S. caramel	14.1
			20 pounds pressure			
10	42	81	48	V. f. brown	S. flat	12.5
15	99	139	63	F. brown	Routine	14.2
20	114	84	193	L. brown	Routine	14.3
25	50	46	80	Brown	S. caramel	14.1
30	78	150	149	Brown	S. caramel	13.7

* Millions per cc.

† Transferred from previous day.

M. = Medium. V.f. = Very faint. F. = Faint. L. = Light. S. = Slight. D. = Dark.

were made for each increment of time under the different pressures employed. The results are included in table 115, the experimental error for plating being ± 14 per cent.

Assuming that the third transfer in any set of flasks sterilized for a given time at a given pressure yields the most reliable data, since it permits of a great degree of acclimatization, the figures under "third day" are of particular interest. "Under 15 pounds pressure" it will be seen that 20 minutes gives the highest counts, i.e., 195 million per cubic centimeter. The same pressure at 15 and 25 minutes also gives high counts. However, the counts for sterilization of more than one-half are strikingly lower. The data are not so consistent for the "first and second days" although in general it is evident that sterilization beyond one-half hour reduces the count. At 30 minutes the count is highest for the first and second day. The color of the milk varied from very faint brown at 15 minutes up to the darker shades at 50 and 60 minutes. As compared with the routine "sour milk" taste, the flavor at the latter periods of time was that of caramel. The acidity as noted in the last column did not show very much variation. From these data it would appear that milk sterilized at 15 pounds pressure should be exposed for at least 20 minutes and not more than 30 minutes before inoculation with *L. acidophilus*. This corroborates the recommendation of Rettger and Cheplin (144).

The data under "20 pounds pressure" indicate that 20 minutes exposure is most favorable. The color is satisfactory and the milk is palatable under these conditions. Despite the inconsistencies which must be expected in such work it is nevertheless clear that a high degree of sterilization yields most desirable results. However, it is inadvisable to extend this period of sterilization beyond 30 minutes at 15 pounds pressure, or 20 minutes at 20 pounds pressure. Our experience has led us to adopt the latter conditions as most satisfactory for preparing *L. acidophilus* milk.

In considering the question of whether the action of *L. acidophilus* is a strictly bacteriologic phenomenon, it was necessary to kill all the viable *L. acidophilus*, in order to determine whether the end-products of bacterial activity or the actual living bacteria were of greatest importance. The problem therefore, was to ascertain the temperature and time of exposure necessary to kill the living organisms, which would cause the least alteration in the chemical composition of the milk. Since it was necessary to have some practical means of effecting this under ordinary laboratory conditions, the Arnold steam sterilizers were used as follows:

One litre portions of skim milk containing 240,000,000 *L. acidophilus* per cubic centimeter in 1½ litre Pyrex Erlenmeyer flasks at 35°C. were placed in the Arnold steam sterilizer for different periods of time. The temperature of the contents of each flask was recorded at the end of the heating period, and represents the maximum temperature attained. The temperature during heating therefore, was gradually rising from 35°C. initially to the maximum. After exposure the flasks were immediately cooled to room temperature and the contents plated in triplicate on whey agar. The flasks which showed no viable organisms were then incubated for 24 hours at 35°C. and the contents again plated to insure sterility. The acidity of the milk in all flasks was neutralized with normal sodium hydroxide prior to the sterilization.

In table 116 are included the results of the effort to pasteurize *L. acidophilus* milk. It is evident that the organisms were not all killed until a temperature of 81°C. was maintained for 10 minutes. Naturally, all exposures more severe proved equally detrimental to the living organisms.

TABLE 116

Pasteurization of L. acidophilus milk

TEMPERATURE	TIME	NUMBER OF L. ACIDOPHILUS PER CUBIC CENTIMETER	COAGULATION OF MILK
		I	
°C.	*minutes*		
32	9	Numerous	+
50	20	Numerous	+
55	20	Numerous	+
59	5	2,500,000	+
81	10	–	+
86	15	–	+
86	20	–	+
73	30	–	+
96	30	–	++++
121	10	–	++++
		II	
81	10	–	–
86	15	–	–
		III	
81	10	–	–
86	15	–	–
		IV	
81	7	20,000	–
81	10	–	–

However, the higher temperatures caused extreme coagulation of the milk as shown by ++++ (see table 116) and were therefore undesirable. It will be seen under parts II and III of this table that the previous results in part I were again obtained under the same conditions. Finally, in order to determine the time of exposure more closely, the flasks were heated during a period of 7 and 10 minutes respectively at the end of which time the maximum temperature was 81°C. The former time was insufficient to effect complete killing and therefore the latter period was adopted. In this way it has been possible to pasteurize several litres of *L. acidophilus* milk daily in the laboratory. Before feeding this milk, however, lactic acid is added in an amount equivalent to that present before neutralization.

Since heating changes the chemical composition of the milk, an effort was made to kill the *L. acidophilus* by freezing. Portions of *L. acidophilus* were therefore placed in a refrigerator at about −3°C. for one week. The milk was frozen throughout this period except for the time necessary to obtain samples for plating, when it was warmed only enough to remove 10 cc. The milk was plated in triplicate every other day. Originally the milk contained about 50,000,000 viable *L. acidophilus* per cubic centimeter. After 2 days at −3°C. this was reduced to 1,000,000 per cubic centimeter. After 4 days the count was 500,000 per cubic centimeter, and after 6 days it was 25,000 per cubic centimeter, or a reduction of 99.95 per cent of the original number present. Complete pasteurization however, was required. Unfortunately, it was impossible to conduct this study further, since the proper temperature conditions became unavailable.

The results here presented cannot be regarded as final, since they are more or less empirical in nature. However, because of the increasing interest in *L. acidophilus* it was believed that they might prove of some service to others working along these lines.

SUMMARY

1. The number of viable organisms in *L. acidophilus* milk is greatly reduced by ordinary ice-box temperatures (9°C.). After 3 days the count is reduced about 90 per cent. When held at −3°C. for 6 days the number of viable *L. acidophilus* was reduced 99.95 per cent. It is more desirable to keep *L. acidophilus* milk at room temperature.
2. On the basis of comparative tests it was found that milk to be used for inoculation with *L. acidophilus* should be sterilized in the autoclave at 20 pounds pressure for 20 minutes, or at 15 pounds pressure for 20 to 30 minutes. Under these conditions a maximum number of viable organisms is obtained after incubation.
3. *L. acidophilus* milk may be completely sterilized in one litre portions by exposure in the Arnold steam sterilizer for 10 minutes (with a final temperature of 81°C.).

Part III

THE USE OF MODIFIED GRAM STAINS[4]

The Gram stain continues to be an ever fruitful subject for experimentation. An examination of the literature reveals a variety of methods many of which have proven valuable. The Society of American Bacteriologists, through the reports of its committee, has offered two methods for consideration which have been widely used (48). More recently, however, two important modifications of the Gram stain have been advanced. The first of these by Atkins (4) proposes the use of aniline sulphate in place of aniline, and a stronger iodine solution, to which sodium hydroxide has been added. The other new method is that described by Burke (27). Here the chief points of interest are the use of: a 1 per cent aqueous solution of dye, sodium bicarbonate, and acetone as a decolorizer. Both Atkin's (4) and Burke's (27) methods have yielded satisfactory results in our hands, and it would be difficult to enter into a discussion of the relative merits of these methods without previous exhaustive investigation. Suffice it to say that we have taken the liberty of combining some of the principal features of each of these methods in an attempt to economize time and expense without sacrificing the quality of the results. Before advancing the reasons for choice it would be appropriate to present the method in detail step by step as used by us.

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[4] Reprinted from Jour. Inf. Dis., *31*, No. 5, November, 1922, 480–482. and State Hospital Quarterly (N. Y.) *10*, August, 1925, 590–593.

1. Air dry a thinly spread film and fix with least amount of heat necessary to kill the organisms and fix them to the slide.
2. Flood slide with dye solution. This is prepared by previously mixing in a beaker about 30 drops of a 1 per cent *aqueous* solution of methyl violet 6B (Coleman and Bell) with 8 drops of a 5 per cent solution of sodium bicarbonate. Allow the mixture to remain on slide 5 minutes or more.
3. Flush off the excess stain with the iodine solution and cover with fresh iodine solution for 2 minutes or longer. The iodine solution consists of 2 grams iodine dissolved in 10 cc. normal sodium hydroxide solution, to which is then added 90 cc. of water.
4. Drain off the excess iodine solution, without blotting (no water being used) but the film is not permitted to become dry.
5. Add acetone (100 per cent) drop by drop until no color is seen in the drippings from the slide, which is slightly tilted. This usually requires less than 10 seconds, and should be reduced to a minimum.
6. Air dry the slide.
7. Counter stain for 10 to 30 seconds with 0.1 per cent aqueous solution of basic fuchsin.
8. Wash off excess stain by short exposure to tap water and air dry. If slide is not clear immersion in xylol has been recommended.

The advantages to be derived from such a procedure are considered separately in the papers of Atkins (4) and Burke (27) and may be briefly enumerated as follows:

In step 2 mentioned above a 1 per cent aqueous solution of violet (either methyl 6B, crystal, or gentian violet) is easily prepared and has relatively good keeping qualities. Sodium bicarbonate neutralizes acidity and improves the intensity of the stain in the Gram-positive organisms. We have found that the more intimate mixture of sodium bicarbonate and dye solution prior to application yields somewhat better results than when these are used separately. A stock solution of dye and bicarbonate cannot be made up since deterioration sets in rapidly.

In step 3 in this iodine formula, sodium hydroxide is used to dissolve the iodine. This is not only economical but also yields free hydroxyl-ions which may aid in intensifying the stain.

In steps 4, 6 and 8 it will be noted that we do not use blotting paper at any stage in the procedure because it not only appears to be superfluous but may frequently leave fibres on the slide, which are undesirable.

In step 5 acetone has proven to be more satisfactory than either 95 per cent or absolute alcohol and is also more economical as regards expense and quantity.

In step 7 we prefer fuchsin as a counterstain because it is bright and gives an excellent contrast. Fortunately in this procedure there is no danger in overstaining when prolonging the applications of stain or iodine beyond the accepted time limits, provided that actual drying does not occur. This does not apply to the counterstain. Control Gram-positive and negative organisms are always employed.

The Gram stain procedure described has been used with particularly gratifying results in connection with our studies on the therapeutic effect of *L. acidophilus* (105) in normal and psychotic subjects (99, 100, 105). The daily routine required the staining of *L. acidophilus* in milk. Using the Sterling modification of the Gram stain with alcohol as a decolorizing agent, the debris was dark purple which made it somewhat difficult to identify individual bacilli. However, with the procedure here outlined the debris is pale pink in color which causes the Gram-positive bacilli to stand out sharply. Furthermore, the Gram-negative debris does not obscure the presence of Gram-negative organisms as Gram-positive debris is likely to do. Again the routine examination of feces in these studies was attended with the usual difficulties when the Sterling modification of the Gram stain was employed. The microscopic examinations therefore were greatly facilitated by employing the present procedure, which not only rendered the debris Gram-negative but yielded a sharper differentiation between Gram-positive and Gram-negative organisms. Thus for the purposes at hand, this procedure proved to be invaluable in point of differentiation, as well as in economy of time and expense.

In order to widen the scope of its application a number of common pathogens and saprophytes were stained by this method. They comprised the following species[5] named in accordance with the latest authorized classification (184); *Neisseria gonorrheae; Corynebacterium diphtheriae; Bact. paratyphosum; Bact. coli; Mycobacterium tuberculosis; Diplococcus pneumoniae, Type 1; Streptococcus viridans; Staphylococcus aureus; Clostri-*

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[5] We are indebted to the Research Laboratories of the Board of Health, New York City; the Department of Bacteriology of the Army Medical Museum, Washington, D. C.; and the Out-Patient Department of St. Luke's Hospital, New York City for the bacterial cultures so kindly placed at our disposal.

dium aerogenes capsulatum; Lactobacillus acidophilus; Lactobacillus lactis-acidi; B. mycoides.

It was found that the above organisms were stained in a way that left little to be desired with regard to the Gram phenomenon or morphology. Particular attention may be directed to the clear cut results obtained with *Neisseria gonorrheae* and *Cornyebacterium diphtheriae,* which ordinarily present considerable difficulty in Gram staining.

SUMMARY

1. A method of Gram staining has been outlined which is based upon the satisfactory results obtained by the use of modifications devised by Burke (27) and Atkins (4).

2. The above method has yielded particularly good results in staining milk slides for *Lactobacillus acidophilus* and in staining fecal specimens. By this method, *Neisseria gonorrheae* and *Cornyebacterium diphtheriae* likewise are well differentiated and more easily identified than by the older methods. The same was found to be true for a number of common pathogens and saprophytes studied.

INDEX OF AUTHORS

Albu, 9.
Albus, 22, 23.
Asayama, 117.
Atkins, 204, 206.
Aznar, 18.

Baar, 118, 119, 120.
Backman, 8.
Bahrdt, 12.
Barney, *see* Cheplin, 29.
Bass, 18, 19, 179, 196.
Bassler, 20.
Baumann, 117.
Beerman, *see* Kopeloff, 15, 32–38, 107–116, 203–206.
Beifeld, *see* Bahrdt, 12.
Belonovsky, 4.
Berry, *see* Cruickshank, 22.
Biernacki, 8.
Blatherwick, 21, 174.
Blumenthal, 118.
Borden, 119.
Breidigam, 23.
Brieger, *see* Baumann, 117.
Broadhurst, *see* Winslow, 205.
Brown, *see* Tisdall, 22.
Bruce, 119.
Buchanan, *see* Winslow, 205.
Bunting, 25.
Burke, 204, 206.

Cahn, 12.
Cannon, 16, 17.
Chang, *see* Breidigam, 23.
Cheney, *see* Kopeloff, 39, 205.
Cheplin, 17, 18, 19, 26, 27, 29, 43, 47, 97, 132, 194, 199.
Cippolina, 13.
Clark, 18, 19.
Cleghorn, 119.
Cohendy, 2, 3.
Cole, *see* Hopkins, 117.
Coleman, 13.
Combe, 1.
Conn, 204.
Coriat, 119.
Croll, *see* Kast, 159, 167.
Cruickshank, 22.

Daland, 119.
Daremberg, 120.
Davenport, 122.
De Gaspari, 15.
De Schweinitz, 117.
Distaso, 6.
Dragstedt, C. A., *see* Dragstedt, L. R., 17.
Dragstedt, L. R., 17.

Ebaugh, *see* Julianelle, 19.
Eggston, 18, 19.
Eisenstadt, 8.
Ellinger, 118.

Fine, *see* Myers, 122.
Finkelstein, 12.
Fischer, 23.
Fisher, 15.
Folin, 118, 119, 154.
Frost, *see* Conn, 204.
Fulmer, *see* Cheplin, 29.

Goldman, 20.
Gompertz, 17, 18.
Grigoroff, 1.
Grundzach, 9.

Harding, *see* Conn, 204.
Hatch, *see* Howe, 24.
Hauer, *see* Kendall, 23.
Hawk, 154.
Herter, 7, 9, 11, 117, 120.

Hines, 22.
Hirschler, 8, 120.
Hitchens, 30.
Holm, *see* Albus, 22, 23.
Hopkins, 117.
Horton, *see* Rettger, 26, 121.
Houghton, 118.
Howe, 24.
Howland, *see* Richards, 120.
Hudson, 21.
Hull, 26.
Hurst, 119.

Jacobson, 12.
James, *see* McIntosh, 24.
Jimenez, 18.
Jones, R. E., *see* Rettger, 26.
Jones, W. E., *see* Bass, 18.
Jötten, 25.
Julianelle, 19.

Kahn, *see* Torrey, 13.
Kapsinow, *see* Underhill, 120.
Kast, 159, 167.
Kendall, 7, 9, 11, 13, 23, 120.
Kerr, *see* MacNeal, 121.
Kirby, *see* Kopeloff, 39.
Kirkpatrick, *see* Rettger, 26.
Kligler, *see* Conn, 204.
Klotz, 6.
Kopeloff, 15, 32–38, 39, 107–116, 203–206.
Krauss, 8.
Krumwiede, *see* Winslow, 205.
Kühn, *see* Luerssen, 5.
Kulka, 7.
Kulp, 29, 159, 167.

Latzer, *see* MacNeal, 121.
Lazarus-Barlow, *see* McIntosh, 24.
Leva, 5.
Levine, 119.
Logan, 12.
Long, *see* Blatherwick, 21, 174.
Luerssen, 5.
Lutz, *see* Bassler, 20

Mackee, 117.
MacNeal, 121.
Matson, 119.
Matthews, 117.
McIntosh, 24.
McNease, *see* Cannon, 17.
Metchnikoff, 1, 29.
Mizell, 18, 19.
Morgan, 119.
Moro, 11, 12.
Morriss, 19.
Myers, 122.

Natonek, 117.
Naujoks, 25.
Norman, 18, 19.

Oehler, 6.
Osler, 119.

Parr, 20, 21.
Passini, 12.
Perry, *see* Clark, 18, 19.
Perroy, *see* Daremberg, 120.
Pilcz, 119.
Pope, 18.
Porter, 118.
Post, *see* Cheplin, 29.
Prucha, *see* Conn, 204.

Rahe, 7, 13, 17, 22, 32.
Reddish, 18, 19.
Rettger, 8, 9, 12, 13, 17, 18, 19, 24, 26, 29, 43, 47, 97, 120, 121, 194, 199.
Richards, 120.
Robinson, 21.
Rodella, 12.
Rodriguez, 24.
Rogers, *see* Winslow, 205.
Rotch, 13.
Rovighi, 8.

Schiller, *see* Distaso, 6.
Schmitz, 9.
Segal, 117–176.
Severin, 6

Short, *see* Kast, 159, 167.
Sierakowski, 24.
Simpson, 121.
Sisson, 14.
Sittler, 12.
Slanetz, 23.
Smith, A. H., 159, 167.
Smith, G. H., *see* Winslow, 205.
Smith, R. P., 20.

Taistra, 174.
Taubert, 119.
Tisdall, 22, 122.
Tissier, 12, 106.
Torrey, 13, 121.
Townsend, 119.

Underhill, 117, 120, 121.

Vorhaus, *see* Gompertz, 17, 18.

Wakeman, *see* Herter, 117, 120.
Warbrick, 118.
Webster, 14.
Weiss, 13.
Wejnert, 4.
Winslow, 205.
Winternitz, 9.
Wiseman, *see* Cheplin, 29.
Wollstein, 12.

Zajdel, *see* Sierakowski, 24.

INDEX OF SUBJECTS

Acidity, of *L. acidophilus*, 22, 24, 45, 115, 172.
  of *L. bulgaricus*, 115.
  of feces, 2, 17, 21, 27.
  of urine, 21, 122–176.
Acidophilus, description of *L.*, 11.
  clinical results, 13, 16, 17–31, 36–183.
  influence on psychoses, 19, 44.
  investigations reviewed, 12–31.
  keeping quality of milk, 28, 30, 46, 47, 197–203.
  milk, compared with *L. acidophilus* filtrate, 78, 86, 92, 93, 95–98, 100, 101, 112–176.
  milk, compared with *L. acidophilus* paste, 75, 88, 93, 94, 112–176.
  milk, compared with pasteurized *L. acidophilus* milk, 76, 78, 80, 82–87, 96, 98–100, 107–176.
  milk, compared with sterile milk, 83, 84, 107–116.
  milk, dosage, 28, 48, 49, 176–180, 195, 196.
  milk, large scale production, 196.
  milk, preparation, 194–196, 199–203.
  milk, temperature for keeping, 28, 30, 46, 47, 197–203.
  nature of therapy, 107–116.
  permanence of results, 53–55.
  recovery from feces, 11–13, 15, 16, 18–21, 27–31, 36–38, 42, 43, 46, 47, 60–106, 122–148, 165–170.
  versus *L. bulgaricus*, 15, 23, 26–38.
  viable numbers in milk, 28, 45, 48, 49, 199.
Antecedents of *L. acidophilus* therapy, 1–10.

Bibliography, 184–194.
Bulgaricus, description of *L.*, 2.
  failure to implant, 6–8, 15, 20, 26–38.
  in American experiments, 7, 8, 12, 15, 26–38.
  in European experiments, 2–6.
  versus *L. acidophilus*, 15, 23, 29, 32–38.

Commercial preparations of *L. acidophilus*, 179, 180.
Critical review of *L. acidophilus* investigations, 11–25.

Diarrhea, causing acid feces, 21.
  treated by *L. acidophilus*, 17–19, 29, 41, 49, 55–59.
Diet, during *L. acidophilus* treatment, 162–167, 178.
  influencing urinary indican, 162–169.
Dosage, 195, 196.

Equipment, 195.

Feces, analytical methods, 196.
  changes in, 21, 28, 29, 36, 42, 55, 60–106.
  microscopic versus plate counts of *L. acidophilus*, 28, 46, 103.
  recovery of *L. acidophilus*, 11–13, 15, 16, 18–21, 27–31, 36–38, 42, 43, 46, 47, 60–106, 122–148, 165–170.
  recovery of *L. acidophilus* after treatment, 37, 105, 106.

Gram stain, method, 203–206.

Indican, urinary, acidity, 21, 122–176.
  in constipation, 8, 122–176.
  influenced by *L. acidophilus*, 121–176.
  influenced by diet, 162–169.
  influence on psychoses, 170.
  method, 122–124.
Intestinal flora, in diarrhea, 22, 29.
  transformation of, 1, 9, 13, 16, 19, 21, 26–31, 36–43, 48–176, 181–183.
  transformation, as influencing constipation, 17, 19, 28, 29, 105.

Lactose, 2, 3, 8, 9, 16, 19, 21, 22, 23, 26–31, 38–42, 48–53, 60–106, 125–183.
Large scale production of *L. acidophilus* milk, 196.

Maltose, a differential criterion, 22, 30.
  fermentation by *L. bulgaricus*, 2, 30.
Methods, equipment, 195.
  for fecal analysis, 196.
  for Gram stain, 203–206.
  for indican, 122–124.
  for plating *L. acidophilus*, 33, 194.
  for preparing *L. acidophilus* milk, 19, 194–196, 199–203.
  for preparing whey agar, 195.
  for urinanalysis, 33, 194.
Motility of intestinal tract, 43, 54, 55.

Practical recommendations, 176–180.
Preparation of *L. acidophilus* milk, 194–196, 199–203.
Preparation of whey agar, 195.
Psychoses, significance of *L. acidophilus* in, 19, 39, 44.
  significance of indican in, 170.
Putrefaction, controlled by *L. acidophilus*, 9, 13, 16, 17, 19, 26–31, 36–43, 48–176, 181–183.
  controlled by carbohydrates, 8, 9, 13, 16, 21.
  controlled by milk, 8, 9.

Rectal administration of *L. acidophilus*, 48, 55, 85, 95–97.

Sterilization of milk for inoculation, 194–196, 199–203.
Summary, 10, 25, 31, 38, 47, 48, 53, 55, 58, 59, 106, 116, 175, 176, 180.
  general, 181–183.

Temperature studies on *L. acidophilus* milk, 197–203.

Unsolved problems, 115, 116.
Urinary acidity, 21, 122–176.
  analyses, 122–124.
  indican, 8, 122–176.

Weight, gain in, 19, 43, 58.

Yale contributions, 26–31.

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